NATIONAL INSTITUTE OF ALLERGY AND
INFECTIOUS DISEASES

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DEFENSE THREAT REDUCTION AGENCY
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FILOVIRUS ANIMAL MODEL

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WORKSHOP

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WEDNESDAY
SEPTEMBER 12, 2007

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The Workshop met in the Main Auditorium in the Natcher Conference Center, National Institutes of Health, Bethesda, Maryland, at 8:30 a.m.

PRESENT:

RENATA ALBRECHT, M.D., FDA
SINA BAVARI, Ph.d., USAMRIID
MIKE BRAY, M.D., MPH, NIAID
PING CHEN, Ph.D., NIAID
MARTIN CRUMRINE, Ph.D., NIAID
ROBERT JOHNSON, Ph.D., NIAID
FRANCIS PELSOR, Pharm.D., MS, FDA
DOUG REED, Ph.D., USAMRIID
TONY SANCHEZ, Ph.D., CDC
BARBARA STYRT, M.D., M.P.H., CDER, FDA

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P-R-O-C-E-E-D-I-N-G-S

8:32 a.m.

DR. CHEN: This morning we will start with our Session III, Filovirus Therapeutics, background and contrasts from studies of bacterial infections and regulatory perspectives. The moderator will be Tony.

DR. SANCHEZ: I would like to introduce Sina Bavari from USAMRIID who will be talking about Filovirus and viral design and rationale.

DR. BAVARI: You want me to talk into this? I'll try to stay here. The title of my talk is filoviral design and rationale. I'm Sina Bavari from USAMRIID. Yesterday during Alan Schmaljohn's talk he mentioned -- he gave a good overview of all available vaccines.

One of the vaccines that he talked about was the virus-like particles. However, his chart was a little bit outdated so I thought maybe I go through some of the newest data that we have for the virus-like particles first. I do recognize that this is a therapeutic session.

However, I thought I should say something about the vaccine ability of virus-like particles also. Our general philosophy is try to understand as much as we can about the biology of filoviruses therapeutically or by vaccines that can counteract that.

Known properties of the viruses so the requirement for viral assembly guided us to actually discovery of virus-like particles as vaccines. I'm just going to spend a couple of minutes going through some of that data. I see they have already started my time so I will go a little bit faster.

The virus-like particles that we are working with are very similar morphology to live viruses and they are fully enveloped. They are not replicating. They generated in mammalian or insect cell-wise so we can actually generate large quantities of them.

You heard a lot about vectors and vector immunity. Well, there are no such thing in the case of virus-like particles because they are not vector based. They generate innate, humoral and cellular immunity. Safely and effectively have been administered in humans as has been shown by papillomavirus, hepatitis B, and Norwalk.

These are actually platforms for delivery of other cargos such as multivalent VLPs. They can carry viral or bacterial antigens. We have shown that we can actually protect against multiple agents by using virus-like particles such as anthrax, SEB, and so on.

These are great tools actually to dissect immunology of filoviruses.

The way we have been making these we transfect GP, NP, and VP40 of filoviruses both for ebola and Marburg. We actually have a lot of slides showing how these virus-like particles bud from themselves. They do get into dendritic cells and if you dim the light a little bit maybe you can see this a little bit better. This has been published so I'm just going to go through it a little bit quickly.

The mature human dendritic cell is critical for initiating a robust immune response as you can see with HLA-DR, ABC, and CD83 model. This data has been published. First we've got the filovirus-like particles into mice and we show that ebola virus-like particles protect 100 percent of the mice and protected 100 percent of the guinea pigs.

We found out the mechanism of how these filovirus-like particles work is not dependent on porphyrin. However, it's dependent on CD4 and CD8 responses. This has all been published so I'm going to go through them a little bit quickly.

This is not published. We wanted to see

if we get a cross protection if you vaccinate one. For example, of you make Musoke base virus-like particles will it protect against Ravn or CI67. The answer is yes and you get a robust response. The response is always 100 percent in guinea pigs.

Then the next question was maybe do the harder studies first. We wanted to see if they protect against the robust challenge when we vaccinate nonhuman primates. We set up a study which a lot of us were talking about yesterday, the interferon studies. What they did they made virus-like particles of ebola, virus-like particles of Marburg, mixed them together and vaccinated nonhuman primates three times and challenged either with ebola or Marburg.

The surprising thing was that after the second vaccination the antibody titers were maximal so the ebola virus-like particles fully protected in nonhuman primates you can see here. The naive animal died with seven days and this was very typical. The VLP vaccinated animals all survived. This was n of 5. We repeated this again and got similar data. The vaccination was both ebola VLP and Marburg VLP and the challenge was 1,000 pfu.

In the next set of studies we went ahead and challenged the animals this time with Marburg. As you can see, the control died and we got 100 percent protection in vaccinated mice that were vaccinated with Marburg virus and ebola virus combined together.

In summary of the VLP vaccination the vaccination with Marburg or ebola VLP protected mice and guinea pigs. Vaccination with Musoke VLP protected against multiple Marburg viruses. Nonhuman primate vaccination with ebola and Marburg VLPs combined together robust antibody titer and maximal after two vaccinations and protected 100 percent of the primates. There was no demonstratable interference of this.

Actually, demonstrated also that we got a robust CD8 epitope specific responses also. All in all I think the VLP is probably on par with any other platform that is out there but is non-vector based.

Now I can move on to why I was invited here, to talk about developing therapeutics for filoviruses. Our general strategy has been that we can either target the pathogen. We can define effective molecules. We can actually do a lot of what I call meganomics. This is combination of a lot of proteomics, bioinformatics combined together to give us targets that we can actually try them.

We look at immune evasion mechanisms and we try to target those. We don't go after compounds or methods that are not druggable so we stop at that point. We target common microbial pathways or viralist mechanisms. On the other hand, you can target the host also.

How do we target the host? We can identify druggable innate immune responsive pathways that can be targeted by small molecules, siRNA, antisenses and so on. We try to enhance mechanism of adaptive immune responses.

We go after pathway discoveries so that means what are the pathways that several viruses use tend to have a single therapeutic or several enveloped viruses for example. We have done that successfully. We go after host pathogenic responses also that is not good so it can be down regulated some of the responses.

How do we do these? The therapeutics that we selected to use this has been going on for almost six years. We use siRNA antisenses or small molecule nonparetic. This is actually a figure that Gerard Iman produced almost seven years ago. In reality it hasn't really changed yet.

That really shows what you can target in filovirus life cycle. There is a fusion process that can be targeted. There is uncoding, there is assembly and budding. Every one of these steps can be targeted, either the host or the virus.

Going back to our general philosophy about therapeutics, we really need to know all the sequence information as possible if you are going to hit these bugs with antisenses or siRNA based therapeutics. We really need to understand as much as possible about all the protein network so we can actually drug them.

One way that we have been doing this is by true large-scale proteomics. We have been taking virions from ebola or Marburg. They get digested 2D gel. You get mass spec data. From the mass spec data you can figure out that all the proteins that you would expect from the virus itself should show up so this is in case this is for ebola.

The host cell proteins that you should be able to see such as TSU 101 that attracts VP40 of ebola, that should show up here also and it did. It found it amongst a lot of other protein. We have done the same thing for Marburg and now we are trying to figure out what the overlaps are. At the same time we are trying to use siRNA and other methods to knock these down to see that each one of these becomes therapeutic targets for us.

Then we, of course, find interactions and the interacting molecules themselves become targets also. This is set up for CD28. That is not a target but I just put that down as an example.

Our general strategy for what we call designer drugs so these are siRNAs and antisenses to actually target sequences within ebola Marburg genome, find out the efficacy of these siRNAs or antisenses by staining cells. In this case we are using live GFP virus that was given to us by CDC. Then we take this into the rodents. The rodents are nonhuman primates. Of course there's a cycle here but I don't have a lot of time to talk about. This is optimization and this is not any different than small molecule design.

I would like to go through and I'm going to just brush on a lot of the data so it's not going to be really deep and maybe during the panel discussion if there are any questions I will be delighted to answer them.

Initially I would like to go through some of the antisense work that we've been doing. These are noncharge antisenses. They are referred to as phosphordiamadite morpholina-oligonucleutides. They are uncharged. The way the mechanism is they stop ribosomal assembly so you stop protein synthesis.

The advantage of the new antisenses are they are uncharged, very stable, unusually stable. There is no interferon responses that you can detect. This data has been published. We did a lot of prechallenge at first to sort of maybe warm us up to antisenses and in the prechallenge study we were able to actually figure out which one of these viral proteins can easily be targeted.

We started a list of them and we actually narrowed them down to VP35, L and VP24. This is a nice dose response of the PMOs starting at 500, 50 and 5. They were given 24 hours before challenge and four hours after challenge. As you can see we can achieve with only two doses 100 percent survival.

We took the same type of PMOs into nonhuman primates. This was published, I think, last year in PLO Pathogen. We are sure that the pre-challenge administration of ebola specific PMOs can protect primates also. We have moved on from dealing with these type of PMOs to dealing more with the charged molecules. These have four, five, or six different charges on them.

From here to here we have actually been dealing with other ways to deliver antisenses also such as targeting them with penetrating peptides. I'm just going to show you a single slide to tell you that we have a lot of expertise in developing therapeutics and how we are actually doing this.

On the top you can see this was no virus. If the lights were down maybe you can see it a little better. You put the GFP virus and you clearly can see the virus infected a lot of the cells. The untagged molecules really don't protect that much while the tagged molecule does dependently protect.

As I was saying, we wanted to really move beyond things that I think clinically may not go for or they are really some TOX data that may stop us later on so we decided to really go with a PMO+. I forgot to mention but our partner in these studies have been AVI, Antiviral Incorporated in Corvallis, Oregon. I've been working with them for almost four years so the data that you see is really narrowing down from four years of the data.

Here we did a post-challenge treatment of nonhuman primate with PMO+. Initially we challenged five rhesus macaque with 1,000 pfu. We treated four of these rhesus macaque with VP24 and VP35 combination. We started the treatment one hour after infection because we thought that this was maybe the bar that was set up previously before us so we stated this. They are going to continue to move beyond one hour.

We continued the treatment daily for 10 or 14 days. The treatment was given subcue and IP. Half a dose was given subcue. The other half was given IP. The total dose was 20 ml/kg. In the first set of studies that we did the naive animal died quickly. We actually had one animal surviving and then died. This is about 75 percent protection. I think this is probably the best that is out there right now.

If you look at the viral titer, and this is, I think, a combination of five naive animals that were challenged with ebola. You can see the viral titer is skyrocketing up to 20 to the 8th quickly. Even the one that died you see a huge lag. It didn't spike as much. The other three survivors they had no viral titer that we could detect. This was done in serum.

We repeated this study and using another set of monkeys and here we treated the monkeys with the same doses of PMOs given half subcue and half IP but we stopped at day 10. In the last study we went to day 14 and here we stopped at day 10.

We lost two of the monkeys and we are not exactly sure was it because we stopped at day 10 or there is some other complication. I'll show you the viremia data. Even the two that we lost they actually had no virus titer in sera the days that they died. We think that they died from complication of ebola infection.

Again, this is combination of several animals and this is for the naive. For the therapeutically treated ones they spiked viral titer by day seven. It went away by day 10 but the animals died on day 15 and 16. We can't figure this out right now. This is the data that actually literally was done a few weeks ago so we don't have all the pat data. I would love to share that with you. It's really interesting to figure out why these animals died.

Again, we are not really given a lot of adjunct therapeutics so we barely gave them saline here and there. We should be given them a lot of other combination therapeutics such as antibodies, such as NAPc2 and so on. I think this actually opens up the door now for a lot of combination therapeutics.

This is an accumulation of the rhesus macaque that we treated with PMO+. As you can see, one of them died by day 11 or 12. The other two died day 15, 16 without any viral titer. The combination is close to 70 percent, I think, which is pretty good from our point of view because when we started this a couple of years ago I never thought I would be standing here talking to you about this type of data.

We have done the same type of therapeutics using PMO+ for Marburg viruses so this is a mouse model that actually Kelly Warfield just generated. We tested these in mice first. We took it into guinea pigs and we targeted VP24 and NT in this case. It seems like the combination you get a pretty healthy response and we protected almost 100 percent of the mice.

This study was huge like 30 or 40 mice. We repeated these and we took them into guinea pigs which I think is actually a very good model for therapeutics. Anytime that we have done therapeutic studies and showed protection in guinea pigs we were almost replicating the same data in nonhuman primates.

I would not discount mouse model or guinea pig model because I'm not really sure how else we can screen a lot of molecules. Both of them are great tools to dissect immunology and cell biology filoviruses. We are moving into nonhuman primates now, of course, and maybe next time if I get invited again I can talk about that data. Hopefully I'll have a lot more on plaque also.

Let me go through the summary of development for antisenses PMO. Originally we identified three unmodified PMOs that were affected in prophylaxis treatment. We further developed -- further development led us to narrow these down to 2 PMO+s that targeted VP24 and VP35 that are both actually effective in post-challenge treatment. These are the best virally directed -- to my knowledge these are the best virally directed post-exploited therapeutics in nonhuman primates at this point.

We have identified two other compounds targeting VP24 and NP for Marburg viruses. Currently we are actually doing the efficacy studies in nonhuman primates. Ebola plus PMOs are surprisingly safe and if injected into mice at 50 times the doses that were used in nonhuman primates, so at 1 gram per kilogram and we did not see toxicity yet. This needs to be repeated. We need to do really detailed plaque studies. Again, our partner is actually involved doing a lot of these studies. This was a quick tox at USAMRIID.

The future for the PMOs are they are moving with the GMP and GLP tox studies. They are doing efficacy in nonhuman primates of delayed treatment. I'm not exactly sure what delayed treatment means here. Is it one day, two days, or is it going to stay at one hour.

I would like to talk a little bit about our efforts on RNAi. This work has actually been done in collaboration and partnership Alnyam, the leaders in siRNA work. I'm going to go through these quickly. I just want to show you just a bit of the data and then at the end I'll wrap it up.

The ebola siRNA has shown that actually we can reduce titers of ebola by about 90 percent. In this case we transfected 293T cells and infected them the next day. As you can see from no si's or negative si control down to some of these specific si's we can reduce these down. Now we've got far better and more potent si's than this initial observation that is actually a few years old.

The last section of my talk is actually something that we have been working on at USAMRIID for almost six years now. This has really tried to develop small molecule therapeutics against filoviruses and other bio-threat agents. Our hypothesis has been can we actually find small molecules drug-like compounds against filoviruses.

Our approach has been to set up high

throughput assays. This we have been trying to use the GFP ebola virus as much as possible and high content imagers to identify small molecules that inhibit viral replication. We screen chemical libraries, large chemical libraries, thousands of them used in the GFP virus to validate these hits using QRTPCR or platforming units.

We quickly go into animals. We start with rodents and then move into nonhuman primates. We identify the targets and we look to see if the target themself can be altered by other drug-like molecules or by the same one. The more we understand about the target the better we can actually explore the therapeutics.

As I said, this actually initiated a major nonparetic small molecule drug discovery program at USAMRIID a few years ago. We have access to a lot of libraries. The libraries come to USAMRIID. We do in vitro testing, Solvay assay, animal studies. This goes into we hopefully can find some hits or leads. This goes into our partners, National Cancer Institute.

They develop pharmacophore-based hypothesis so we can find better and more potent examples of these by 3D data search mining. Then they propose other compounds for improvement. This improvement goes to organic chemist. He builds the compound and sends it back to USAMRIID. We go through this cycle several times to come up with leads. The data that I'm going to show you next is one round of this chemistry.

The typical ebola virus drug screenings 96-well plate. Cells going to 96-well plate. The infected cells have MOI of 1 with ebola GFP virus. Hopefully you'll get some that are a little less green and some that are white. That means they are fully protected. We let this infection go for about 48 hours. Cells and everything gets fixed. We bring it out of the suite. We run it on high-contact imagers.

This is blurry. I apologize for that. It's not your glasses or it's not mine. Then they analyze the data. These are just examples of ebola inhibitors. All of this work that I'm talking to you about was done actually in collaboration with functional genetics and integrated biotherapeutics. functional genetics is a lead in these studies.

This is examples to show we have several compounds that you can actually inhibit infection at low molecular range We can see the same thing in the plaque forming units also. It can substantially reduce the infection.

We have taken some of these into mice. As you can see if you challenge the mice with ebola they die within 10 to 12 days. That's our saline control. Different compounds show different efficacies, as you would hope, to see. We have few compounds here that we are reproducibly getting 100 percent protection.

This is done post-challenge and we have done a pre-challenge also. We have done it both ways. We started with pre-challenge first and then we move into post-challenge. The amazing thing about these compounds are that they are only given three times. It says something about bio-availability of these compounds. They were given a 5 mg/kg.

Another set of studies we actually wanted to see if the compounds protect even once or twice at only 1 milligram per kilogram. As you can see, some of the compounds even at 1 milligram per kilogram even once can protect. Continuing on this theme of small molecules, the other thing we wanted to do we wanted to see if a single dose -- do we see a dose response.

This is critical for finding therapeutics. It tells you a lot about the drug that you're working with. As you can see, as you increase the dose of the drug at the single dose, you get up to 100 percent protection. This is done over and over again.

Summary of the development of small molecule inhibitors. Overall we have actually tested a lot of libraries. We have probably tested well over 15,000 compounds. I put 12 here to be modest about it. We found a lot of hits. Actually when you look at these hits you find each one of them are distinct scaffold.

We have gone into those scaffolds and we have found a lot of sub-libraries that now we can actually go after. Many of these compounds we believe that they work on host. I don't have data to show you but that's my belief. They work against several pathogens, biopathogens.

We have done a lot of secondary screening using the plaque and QRTPCR. We've done a lot of mouse studies. I'll show you a lot of the data. The ongoing studies are really to search out mechanistic characterization of these and try to do a gas phase pharmacophore.

Since we don't know the specific target we can actually put these molecules together and see based on hundreds of these compounds the ones that work versus the ones that don't work can be dissected from each other and we can learn something about the target by doing that and fine other maybe more potent molecules. We are doing scale-up synthesis for doing guinea pigs and nonhuman primates.

Overall summary of our filoviral therapeutic efforts, the data that I didn't really show you but we have shown that we can actually protect using VLP if it's given innately also so that means a day or two days after infection this protects. The protection seems to be NK mediated. We identified a lot of false factors by proteomic analysis and knocked those down getting a lot of good data out of that.

I'll continuously share that with you. We are targeting common viral processing pathways such as BPS pathway. We have shown this to be actually therapeutically promising. This is a protein sorting pathway. We've done a lot of systemic genomics, genome-wide siRNA screen that I talked to you about and we've got a lot of promising lead sequences in vitro.

We have identified second generation PMOs, PMO+s which protect mice, guinea pigs in Marburg and nonhuman primates in the post-exposure therapeutic against ebola. We have identified several scaffold, druggable small molecules that inhibit ebola replication.

This work could not have been done without direct involvement of this big group here, Kelly Warfield, Dana Swenson, and Javad Aman who has moved on now, and a lot of other people that actually have been constantly helping us with these studies.

I listed some of the names in here but at USAMRIID in general everybody has been extremely positive about our work and fairly supportive, Alan Schmaljohn as I put previous here and Diane Negley, Mary Kay Hart, Bill Pratt, Mike Parka. Really the whole pathology division has been extremely helpful to us.

At NCI Rick Gussio is a leader for our Molecular Modeling and Molecular Structural Based Drug Design. At AVI Pat Iverson and others. At Functional Genetic Mike Goldblatt. At Integrated Biotherapeutic Javad Aman. At Alnyam there's a team that has actually been extremely helpful to us and very supportive.

I could not stand here and talk about any of this data if it wasn't really because of the initial support I got from Defense Threat Reduction Agency and programs that next setup at DOD the TMTI program. A lot of funds actually came from NIH to support the siRNA work.

DR. SANCHEZ: I think we are running a little bit behind schedule so we only have time for a couple of questions if there are any. Please use the microphone.

PARTICIPANT: I have two questions. Maybe you can answer the second one first if you would prefer to. You have shown a lot of data here and also some of the data which VP24 and 35 giving 100 percent prediction. One of the questions I have is your dosage is very high and when you convert that to 70 program man which the DOD standards are, it works out something like between 2 grams to 2.5 grams per day if you are doing it for 14 days. That is one.

The second, there are so many compounds and most of those compounds are showing 100 percent or 80 to 100 percent protection. You have asby or your asby DOD which are the best two candidates you want to send for everyone's development. Which of those will you identify?

DR. BAVARI: Okay. I think I probably will be able to touch on that but I probably won't be able to answer both of those questions. About the doses of PMOs the dose starting here is 20 milligram per kilogram with a PMO+. There is no reason why we can't go lower than that. There is no reason why we couldn't improve upon that. I don't think the dose is going to stay the same.

We started actually at higher doses than this and going down slowly. 20 milligram per kilogram I'm not sure how you can gauge that this is a high dose or a low dose for antisenses because we've gone 50 times over and we haven't seen tox data yet. On the second aspect of your question about how would you narrow down therapeutics?

How would you narrow down your leads into something that can actually move on to phase I clinical trial. I think that was your question. There I think it's really up to our partners. We have licensed these compounds out to functional genetics and I think it depends on functional genetics and, of course, people who fund them to be able to move this forward.

If the funding agency decides that they want to have one and a second one as a backup, that's what we're going to have to do. If the funding agency decides to only take one of them, which one would you take, then I think you have to go back and take a look at the combination of the data. Even post-exposure what would you want? Do you want it to be one-day or two-day post-exposure? I think those type of mandates should be driven by joint requirement offices at both HHS and DOD. I don't know how I can actually answer you better than that.

PARTICIPANT: Two quick questions about the VLPs. In the summer you seem to be saying that you've gotten post-exposure protection using VLPs but I didn't see data. If you could clarify that to me. I'm also wondering in terms of using VLPs potentially therapeutically could you deliver an RNA molecule with a VLP that would be therapeutic?

DR. BAVARI: The data that I showed we have some data in mice that we can come back after a day or two days post-exposure given VLPs and it tends to protect. I didn't show that data. We've done it from three days before, two days before, one day before and then we moved on. That data we think is NK dependent.

Now, what was your second question?

PARTICIPANT: You were talking about -- I don't remember the term, polyvalent VLPs. Anyway, you can carry an RNA molecule from inside. Could that molecule be therapeutic?

DR. BAVARI: I think that would be difficult to do. That would be difficult to do.

DR. SANCHEZ: Okay. We should end there.

The next presentation will be by Renata Albrect from FDA, regulatory lessons learned from ciprofloxacin for anthrax.

DR. ALBRECHT: Good morning. I would like to thank the organizers for inviting me and, yes, I really at this filovirus workshop have been asked to speak about ciprofloxacin for anthrax. What I'll try to cover in the next 20 or 30 minutes is to give you a brief introduction to both anthrax and ciprofloxacin and then speak about inhalational anthrax post-exposure so how cipro is approved for post-exposure prophylaxis of inhalational anthrax.

And then talk a little bit about treatment of inhalational anthrax but, in that case, actually talk about the challenges that are facing us as we evaluate anti-toxins. So by way of introduction, ciprofloxacin was approved for post-exposure prophylaxis of inhalational anthrax on August 30th of 2000 so that's about seven years ago.

The indication was actually listed as inhalational anthrax (post-exposure): To reduce the incidence or progression of disease following exposure to aerosolized Bacillus anthracis. We had sufficient data so that we were able to label this both for adult use and pediatric use.

A little bit about anthrax. It's a bacterial infection caused by B. anthracis. The clinical manifestations can be variable depending on the route of exposure including cutaneous anthrax, inhalational anthrax, which is the indication of interest, as well as gastrointestinal disease.

As you know, bacillus anthracis is a CDC Category A bio-terrorism agent. Historically it's been considered or has been susceptible to penicillins and doxycyclines but there was concern back at the time we were looking at this about bio-engineered strains that might be resistant to these organisms and, therefore, ciprofloxacin was of interest.

The virulence factors of the organism include capsule, protected antigen, edema factor, and lethal factor. Anthrax, of course, in the last century and this century is extremely rare. At the time we were considering this back in 2000 we did not have the information on the single patient from last year and certainly not on the events of October 2001 when 22 cases of both cutaneous and inhalational anthrax were reported.

What we did have available to us was published literature and actually additional literature on the 1979 Sverdlovsk exposure which was an accidental release of spores from a Russian military facility. There were actually over 70 deaths reported in a publication on 42 of those patients where pathology was available so we learned a fair amount about the human disease and its pathology from that series of publications.

A little bit about cipro. Ciprofloxacin is a fluoroquinolone antimicrobial. It was first marketed in 1987 and it's available in oral and IV form. This was all information we already had in 2000. The product is approved for a whole range of infectious disease in humans including respiratory infections, skin, bone, and typhoid fever. The later is noteworthy because it's an infection of the monocyte/macrophage system which is analogous to what we know about anthrax.

In addition, we had a great deal of safety information on ciprofloxacin based on millions of prescriptions given to people. Equally important there was also safety information available for use up to and over 60 days in clinical trials in patients who had either osteomyelitis or children who had cystic fibrosis as well as during actually use. These were all relevant background pieces of information that we had available to us.

So let me now very briefly summarize inhalational anthrax, the post-exposure prophylaxis regulatory decision. How were we able to determine that cipro was effective and efficacious in post-exposure inhalational anthrax? Clearly we couldn't do human studies. There were no patients so, to make a long story short, we turned to the animal model of infection in the rhesus macaque and relied quite heavily on the work done at USAMRIID by Friedlander and others which they published in 1993.

Parenthetically we reviewed other publications and other information on the rhesus and on anthrax and in other animal models but this was the model really that provided the bulk of the information.

Very quickly, this is actually a graph from the publication. This was a study -- six-arm study. We were really focusing on two of those arms. The two arms we were looking at were the saline control and then the ciprofloxacin. The saline control, as you'll see in a while, had a 90 percent mortality. Then the cipro arm, which is the triangle, one of the animals died of anthrax and one due to an accident during gavage administration.

I only mention this because this is actually a six-arm study. Two of these arms were of interest to us. The animals received antimicrobials within a day of aerosol exposure to the spores and then received antimicrobial treatment for 30 days and were followed.

Let me quickly cut to some of the findings that we found persuasive in making our regulatory decision. Fundamentally what I'm going to spend the next several slides doing is talking about the similarities between the nonhuman primate, the rhesus macaque, infection and course compared to the human disease infection and course.

First of all the pathogen. In both the nonhuman primate and in humans it's the same so this was very reassuring. It's bacillus anthracis. In the USAMRIID study the Vollum strain was used. We also have experience with the Ames strain during the events of 2001 and also later on as levofloxacin was looking for approval.

In addition, I didn't mention on the slide we had in vitro susceptibility on the organism, actually on 90 different isolates, to various agents including ciprofloxacin and the MIC was quite low. It was .06 micrograms per mil.

A second similarity was exposure to pathogen. As was discussed yesterday, you can exposure animals to infections via parental routes, intraperitoneal, and others. But the route of interest was inhalational and so, in fact, in both the nonhuman primate and human disease inhalational anthrax the exposure is via aerosol to the lungs and that's what was done in the nonhuman studies. The spores that were administered were approximately 10 times LD50 or five times 10 to the 5th spores to the animals.

Additional similarities between the nonhuman primate and humans the course of the disease and the pathogenesis of the disease in the absence of antimicrobials were comparable. The disease has a rapidly fatal time course in both the nonhuman primate and in humans. Signs and symptoms I have put in parentheses because we don't have that kind of parallel between the nonhuman primate and the human.

Bacteremia and toxemia is present in both and the outcome is similar with low survival and high mortality. All the animals that died, they were, of course, evaluated to see if the death was due to anthrax or some other cause.

Another area of similarity that was quite important to us was drug and drug administration. Of course, the target product for human approval was cipro and that was the product that was administered to the animals. We knew going in what dose we were looking to evaluate in humans because of what we know about ciprofloxacin.

While the whole range of regimens are approved for the various infections. The one of interest was the 500 mg q12 regimen given orally primarily. As I mentioned earlier, the duration was 60 days. In the nonhuman primate study, in fact, the animals did receive ciprofloxacin and via the oral route they were given the product every 12 hours. The dose happened to calculate out to be 125 milligrams per animal.

As far as intervention, it occurred within about a day of exposure so that it was not before the spores had been administered but it also wasn't delayed to the point where the animals were actually bacteremic.

Pharmacokinetic similarities. This ended up being the surrogate, if you will, in which we made the approval decision. Here I have just quickly given the information. So for the adult given the 500 mg dose Cmax levels were approximately 3 mcg/mL, Cmin down to .2 and AUC of about 28. We also had pediatric data with the 15 mcg/mL dose. Finally, we had rhesus macaque data from the USAMRIID study. All of this information was taken into consideration.

Another area of similarities was the outcome. This is based on the course in the controls and comparing that to what we knew from the Sverdlovsk exposure in humans. The disease has a rapidly fatal downhill course. There are some findings grossly anatomically that are comparable such as the mediastinal widening which is the involvement of the hilar lymph nodes by the organism. You can also have meningeal involvement.

I have actually copied slides that Dr. Friedlander presented at the advisory committee that I'll mention a little bit later. The disease untreated is highly fatal and histologically there are also similarities in the findings between the nonhuman primate and the human disease.

These were the numbers from the animal efficacy study that allowed us to conclude that cipro was superior to saline. While 10 animals per arm started, one of the cipro animals died due to a gavage accident. That animal was not infected at the time it was autopsied.

Actually, the animal that is reported as an anthrax death died after ciprofloxacin was stopped so, as I mentioned earlier, it was 30 days of administration. There were actually no deaths during the antimicrobial administration. Very highly significant P value that made us believe that we had evidence of efficacy.

Here is just a summary of the similarities on the various parameters that I mentioned, the pathogen, the route of exposure, the course of disease, drug, drug administration, pharmacokinetics, outcome, and findings. Although that was persuasive to the review staff, we took it one step further which is before making a regulatory decision we actually took this application before an open public advisory committee back in July of 2000, presented all this information, and the committee voted to recommend approval.

With that, we actually did approve this under the Subpart H of the regulations. This is the accelerated rule. I want to mention that because to contrast that with the animal rule that Dr. Abdy discussed yesterday. This is an alternative mechanism to the animal rule.

In the product labeling we actually did

say that ciprofloxacin and sera concentrations achieved in humans served as a surrogate endpoint reasonably likely to predict clinical benefit and provide the basis for this indication.

Part of the regulations then require confirmatory information. During the events following October 2001 we were able to obtain information from much of the CDC work and updated the labeling to reflect that.

Very briefly, as I mentioned, the USAMRIID study was a six-arm study. It did allow us to then further label doxycycline and penicillin with the appropriate doses. Levofloxacin was evaluated in a separate study. Again, this is another fluorquinolone. Let me only mention briefly in the bottom part of this slide one of the challenges we encountered is when I mentioned how dosing is important the adult human dose is 500 QD once a day.

In animals once a day dosing results in extremely rapid clearance so, in fact, the company employed a hollow fibrin model to determine what doses they could provide that would at no time during the dosing interval exceed human exposures to levo so that was a very important consideration. They succeeded in doing that, conducted the study, and levo was also approved for post-exposure inhalational anthrax.

We had less safety data on levo and reflected that in the product approval. The approach then to approval of post-exposure prophylaxis of inhalational anthrax was that we were able to determine that the animal model of infection, the rhesus macaque, and the human infection had extensive similarities of various parameters.

Then based on the study it was shown that the cipro levels achieved in the nonhuman primate were protective. They reduced mortality compared to placebo and they exceeded the MIC of the organism. These levels could also be achieved in humans and they served as a surrogate, as I mentioned earlier, and resulted in a Subpart H approval which is the accelerated approval to be contrasted from the animal rule. That, of course, is another mechanism that we are aware of and is being considered for other approvals of treatments for counter-terrorism agents.

Let me quite briefly talk a little bit now about the other side of the disease spectrum which is inhalational anthrax treatment of the established disease. We do not currently have a product explicitly approved for inhalational anthrax disease. We are actually working through that process now and encountering a number of challenges.

How do we distinguish the treatment of established disease from the post-exposure prophylaxis indication? In our thinking the disease is characterized, we think, by the presence of bacteremia and toxemia in humans. Going back to the events of 2001 11 patients were diagnosed with inhalational anthrax.

Five of those actually did not survive. In those patients antimicrobials were not always sufficient. Necessarily there is the need for antitoxins. We are interested in an animal model of infection that would allow the study of these antitoxins but we do need to identify that model.

We need to be able to determine when the animal has developed the established infection and when do we intervene. That has been challenging. There is no clear program. There is no clear way of knowing exactly when the animal has the infection. As I mentioned, the current thinking is that diagnosis would be made when bacteremia and/or toxemia is present.

So going back to sort of that chart of what are the various parameters we are looking to compare. In the setting of inhalational anthrax established disease, again, the pathogen would be the same. The route of exposure we would expect to be the same aerosolization. The course of the disease we expect to be the same but the big challenge is when do we intervene and this was asked earlier. When do you know that you have disease and you need to intervene with treatment.

As far as product and administration, that which we knew for cipro we don't have that kind of database for some of the products that are being evaluated for treatment of anthrax. Also, information on pharmacokinetics is missing and, as far as outcome, of course, we are looking for the same survival versus mortality.

So there are a number of challenges that we have in terms of evaluating products for the treatment of established inhalational anthrax disease. There have been some publicly presented data in both some rabbit studies as well as nonhuman primate studies trying to determine the time needed for intervention. In fact, because this is a much newer area for us, there is the need for those animal infection models to be repeated so that we have both reliable and reproducible data that can then be taken into studies of the nonhuman primate.

The current thinking is timing is going to be very important. If intervention is too soon, then we are really more in the post-exposure prophylaxis setting. If it's too late in this rapidly progressive disease, animals may die and it may not be possible to show the benefit of an intervention or a treatment.

As I mentioned, before we don't have programs or other markers, at this point the thought is that intervention should be when there is a diagnosis of bacteremia or toxemia made. There are no antitoxins approved and so to be able to have persuasive information in contrast to what we had with cipro we anticipate that we would need two animal species of infection.

Not only would we need two animal species but the types of studies we would need would be studies to demonstrate that the antitoxin is superior to placebo in each of the two species. We also expect that we would need to have studies that evaluate treatment with the antitoxin plus an antimicrobial to really be able to understand what to expect if the product is used in humans since we would expect that antimicrobials would be given along with those products.

Other challenges that have been brought to our attention and are fairly plausible are that these studies are not trivial to do. There are a limited number of animals because we are, as I mentioned, interested primarily in nonhuman primates, although a second animal species will also be needed.

We have learned that there are not many sites that can do these studies. Because this is a fatal or lethal agent clearly a high level of protection for the workers. It is a very resource intensive process as we have learned. There are multiple procedures needed for animals and there is extensive monitoring needed for each animal so we recognize this as still an area that more work is needed on.

Just to summarize what I've talked about, for inhalational anthrax post-exposure we were able to put labeling into four antimicrobial products, ciprofloxacin, levofloxacin, doxycycline and penicillin G and I have briefly summarized that. The regulatory approach under which we were able to achieve that was the accelerated approval Subpart H of the regulations, not the Subpart I animal rule.

In contrast in developing products for the treatment of inhalational anthrax that continues to be a challenge. Because there isn't a product used for other human infections that could also be used for anthrax we are in novel territory. The expectation is that approval will need to be done under the animal rule and there are quite a number of questions that still remain to be answered as we proceed in that.

That's all I have.

DR. CHO: Gary Cho from DTRA. I have two comments. First one, obviously the anthrax and cipro label seems to most of us probably the easiest one to get that label for inhalational anthrax because we have a lot of history for that drug. We understand it better than most other challenges. Obviously you have a lot of safety data there you can do the new indication approval.

I wonder for other bio-agents about defense because we are seeking to approve the drug for the new indication of cipro for inhalational anthrax. Do you think we will be following a similar pattern for what we did for the cipro for some other new drugs coming down? That's my first question or comment.

Second one is, let's put it this way, we keep hearing from FDA people for the animal rule asking for new drug for the bio-agents the bar may be higher than regular but I wonder if you have any further comments on how can we do that in light of this kind of approval. That's probably a hard thing to -- lot harder to do especially when you consider defense emergence nature.

If you raise that bar very high, even higher than regular, I don't know when we can have a production hand. Obviously UA is another road you can do. That is a candidate there, you know, when you push for the further license. I would like to hear more comment from you.

DR. ALBRECHT: So to your first question of whether similar approaches can be used as were for cipro anthrax. I mean, first, I would agree. I think with cipro anthrax we were fortunate because there was so much prior information to begin with. There is so much known about the product.

I think when you are dealing with the product that had been given to 270 million people worldwide you feel like you really understand that. We don't have that for a lot of the products that are being used for treatment of the other counter-terrorism agents except when there are obviously other agents that are susceptible to cipro so that approach can be used for those agents.

I think the answer is yes and no as far as the approach because a lot of the principles that I've tried -- I realize I did it in a very over-simplified manner. A lot of the approaches and elements that I've talked about are really the same ones that come up regardless of the disease. If the pathogen is substantially different or has been adapted, that's going to raise questions in people's mind.

If the route of exposure is different, that's going to raise questions. If the animal is substantially perceived as different from the human disease whether in histopathology or organs affected. All of those are questions. I think as people look at it some people may feel like, "Well, let's use it because it's a fatal disease."

Others will be more skeptical because, as we know, for example, during October 2001 and subsequently, over 10,000 people received the product. As we look at these we think of even worst scenarios of millions of people. You want to have enough assurance that what you are going to be approving is safe and effective as the regulations would have us determine that.

As we have talked internally, the more you have to extrapolate from all of these different components to what you are seeing in the laboratory or in vitro to what you are seeing in the human disease the more people may be cautious to say let's make sure that we are convinced reasonably with some reasonable amount of evidence that we have that.

When you say a high hurdle or higher standard, I'm not quite sure necessarily what the connotation of that was but it sort of goes without saying that in the absence of what we typically talked about as two adequate and well-controlled studies in people, you are going to be making links and that may be what comes up as being that higher hurdle.

In addition, a lot of work has been done in anthrax and other diseases looking at animal models. Those are all surrogates for the actual animal which is the human, the Homo sapiens. It may, therefore, seem like there is a lot more. For those who are familiar, in infectious disease we very often have animal models of infection that are evaluated before the product goes in humans but it's more a proof of concept here as I've outlined it.

There are a lot of details that we try to show how comparable is what we are seeing in the animal and how easy is it to extrapolate to what we expect in humans. I don't know if that got to your question but I think the principles that we used in cipro anthrax I think are principles that would apply to others but it's correct that other and more data will be needed as well.

DR. SANCHEZ: Time for one brief question.

MAJOR ALVES: Yes. I just have a couple comments. I was just going to say traditionally speaking I guess in nonhuman primates when I worked with them with these aerosolized exposures, roughly about four to five days is when you start to see deaths in a lot of these animals with classic gross lesions consistent with inhalational anthrax.

The second thing is I liked the comment that you made that it is difficult to try to set up criteria to determine exactly when it is time to intervene with treatment. Now with these ITS telemetry device systems, I think that is providing a lot of good data on a lot of these animals. However, some of these procedures, telemetry devices and monitoring, they are pretty invasive devices. Under BSL-3 and BSL-4 conditions it's very difficult to monitor so I really like that slide.

The last thing I would like to say is have you seen or have you worked with ciprofloxacin using an IV in these nonhuman primates? The studies you were showing us were pretty much peross but we saw the jackets that Tom Geisbert showed yesterday that they were using under some of their studies. I was just wondering if people were looking at it with anthrax?

DR. ALBRECHT: I think the short answer is no because what we've done is the same way I mentioned, that the surrogate was the sera levels that we know in humans, the pharmacokinetics of both ipro oral and cipro IV, so we actually use that extrapolation. Oral cipro is 80 percent bio-available compared to the IV so the doses were then proportional to that.

But certainly it would have been useful information but given, as you have said, the limitations that wasn't critical. Now, in the penicillin arm that was pen G procaine. Those animals actually received the product IM which is consistent with what's done in humans.

It's the parallel with human and then to the degree we can extrapolate exposure in humans we will try to do that recognizing, or maybe I should say when we don't necessarily believe we need additional animal studies we wouldn't just ask for them but reproducibility is, of course, an important component of all research.

DR. SANCHEZ: Thanks, Renata.

From anthrax we move on to plague. The

next presentation, clinical pharmacological perspectives in dosing consideration of gentamicin for plague given by Francis Pelsor from the FDA.

DR. PELSOR: Good morning. I am supposed to talk about the clinical pharmacological perspectives in dosing gentamicin for plague. I think these perspectives will emerge as I talk about this project we are developing, gentamicin therapy for pneumonic plague under the animal rule.

I need to tell you that the views and information in this presentation are mine and they do not reflect the views and policies of the Food and Drug Administration.

In this presentation what I want to do is really take you through the development of gentamicin using the animal rule. There are four scientific criteria that need to be satisfied to gain approval via this route. The four criteria are referred to as pillars. My area of clinical pharmacology really I focus on the fourth one. I was not able to attend all of the sessions yesterday so I don't know how much you discussed the animal rule.

I will make some remarks about it and if it's redundant, I apologize for that. Then I'll talk about the development of the animal model for pneumonic plague, use of this animal model for dose selection in man, but there's a bit of a twist here.

There is a major issue regarding dose selection for the animal model. That is, what dose do we take in to the monkeys, in this case, to look at efficacy. Monkeys are not an unlimited resource and the facilities that do these studies are not unlimited. Costs are extensive so this is a real consideration.

We need to get the dose close to right when we go into the animal model. Then after we have the information from the monkeys translating this information to humans, I'll talk about some additional in vitro methodology that can help facilitate focusing in on getting these doses right. Then, lastly, some summary and conclusions.

The animal rule is properly titled "New Drug and Biological Drug Products. Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies are Not Ethical or Feasible." For drugs this is Subpart I and they go to regulations of 21 CFR 314 for Biologic Subpart H. The rule was proposed in October of '99 and finalized in May of 2002 and allows for the use of adequate and well controlled animal studies as evidence of effectiveness for approval.

Now, just a couple comments, brief comments really. I won't go into the animal rule in great detail. There are a lot of pieces to it. You should note it can only be used when efficacy evaluations are not feasible under any other FDA regulation. Safety for these products must still be established through the traditional path, animal toxicology and human safety.

The four criteria, and I call them pillars. The first one, that there is a reasonably well understood pathophysiological mechanism of the toxicity and that you understand how to prevent it or reduce it.

Secondly, that the effect is demonstrated in more than one animal species expected to react with a response predictive for humans. That is unless there is a single animal model that represents a sufficiently well-characterized model for predicting a response in humans.

Pillar 3 is that the study endpoint should clearly be related to those of benefits in humans. That is generally the enhancement of survival or prevention of morbidity. The last point, the last pillar, Pillar 4. As I said, this is the street that

I live on. The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allow selection of an effective dose in humans.

Briefly just a little bit about gentamicin therapy for pneumonic plague. Pneumonic plague is caused by Y. pestis, a CDC Category A biological threat agent. Gentamicin has been recommended as preferred therapy for contained casualty situations. The recommended doses are 5 mg/kg IM or IV once daily or 2 mg/kg loading dose followed by the divided 5 mg/kg, that is 1.7 mg/kg three times a day.

Also children, 2.5 mg/kg IM or IV three times daily. This comes out of the JAMA paper by Ingelsby, et al. Human trials of antibiotic efficacy against pneumonic plague are not feasible so this makes an indication for pneumonic plague a candidate for development under the animal rule.

With respect to development of the animal model, the African green money was chosen as the animal model. AGMs are susceptible to Y. pestis through an aerosol route. The AGMs develop pneumonic plague that mimics the human disease. There is an extensive experience at USAMRIID since the early 90s but it remained to be determined the appropriate time for drug intervention in this model.

I'll describe a little bit about the natural history study. With each of the -- this is gentamicin but with each of the drugs that you would expect to evaluate in this model, there is a need to determine the drug toxicity and pharmacokinetics in the African green monkey.

Gentamicin, for example, has been extensively studied in a wide variety of animals and in humans. There is a great deal of information but to this point in time, it has not been, or was not, evaluated in the African green monkey. This is an important point about species and even breed differences. You need to consider that as you look at developing product through the animal model route.

For the natural history study six monkeys were evaluated. They were infected with 100 plus or minus 50 LD_{50} of Y. pestis, the Colorado 92 strain, by aerosol. Measurements were by continuous telemetry. Blood samples were via catheters and clinical signs were monitored. The point here is the incorporation of continuous telemetry monitoring into this study.

We found that overall four of the six animals which became bacteremic did so by 72 hours. Fever was the most consistent early clinical sign of disease. There was no bacteremia or disease in two of the six animals exposed to less than 20 LD_{50}.

On the previous slide I said the target was 100 plus or minus 50 LD_{50}. In fact, in this study there was a broader range of doses that were actually delivered. In future efficacy studies the problems that they had with dosing were resolved so we could accurately control the 100 plus or minus LD_{50}. In this study we didn't.

Now we determined that the appropriate time of intervention was 76 hours after exposure or there was a backup that if the cohort developed a consistent fever greater than 1.5 degrees centigrade above base line for two hours, if the majority of animals developed this level of fever, then treatment would begin. In future studies the 76 hours turn out to be the appropriate time for initiation of therapy. All the animals were treated at that time.

I want to move on to the pharmacokinetics assessments in the African green but I want to get into giving you some terminology first to make sure that you understand what it is that I am talking about when I talk about the pharmacokinetics and the parameters, the pharmacodynamics parameters down the road here.

Pharmacokinetics usually comes from serum concentrations of the drug. Pharmacodynamics usually comes from the minimum inhibitory concentration. We talk about three parameters most of the time when we are dealing with antimicrobials, time above MIC, Cmax to MIC, and AUC to MIC. The time above MIC is simply the time that the concentrations exceed the MIC. The Cmax is the peak concentration to the MIC. The area to MIC is usually a 24-hour area under the curve relative to the MIC.

The gentamicin pharmacokinetics study that we did was in six monkeys, three males and three females. We looked at three dose levels, three mg/kg, 4.5 mg/kg, 6 mg/kg. These were 20 minute IV infusions. There was a week washout between each of the doses. That is, each of the six animals got three doses. Blood sampling was conducted predose, end of infusion, 20, 40 minutes and 1, 2, 3, 6, and 8 hours post-infusion.

This slide shows the mean plasma concentration time curves and the pharmacokinetic parameters. I'll point out that at 3 mg/kg we are seeing concentrations of at least on the average of 17 mcg/mL. Half-life is a little bit over one hour. Maybe average across the three doses of about 1.2

hours. This is a half-life that is much faster than half-life of gentamicin in humans and will be a serious consideration later on when we look at comparing doses in humans versus doses in monkeys.

At the same time that I did the traditional pharmacokinetic analysis, I began to build a population pharmacokinetics model. I knew down the road in the efficacy studies that we would be doing limited or sparse sampling. I wanted to determine some peak concentrations, -- that is, estimate some peak concentrations and estimate some exposures from those sparse samples.

The way that I can do that is through Bayesian methods that are part of this population PK approach. If you compare the values on this slide for clearance and the two volumes, it's very close to the values that you saw on the previous slide and that's as expected.

Now, we also did a toxicity study in the African greens. Again, we used 10 treated monkeys in six controls. These were multiple doses twice daily for 14 days. We divided the 3, 4.5, and 6 milligram doses. We divided them in half for these 12-hour intervals and collected some samples for blood concentration measurement predose at the end of the infusion half hour, one, two, and four hours post-infusion. There was no major toxicity at the 3 mg/kg dose twice daily for 14 days.

This slide now shows an overlay of the observed concentrations out of the TOX study and the predicted values, that is the 95 percent interval of predicted values from our population model. At day 1 and at day 14 there is good agreement between the concentrations that we're seeing in the monkeys in this study and what we predicted that we would see.

Now, moving onto determining a dose to take into the monkey efficacy study. Really where to begin? Traditionally with gentamicin and aminoglycocides the thinking is that if you have a Cmax MIC ratio 10 to 1 that this would be the target. It comes from a classical paper by Moore, et al., from Johns Hopkins where they looked at 188 patients with gram negative infections and they found that at levels of 10 mcg peak to MIC ratios they are seeing about 90 percent plus response rate.

That is the traditional paper. The MICs, I should tell you, for the strain that we were using in the efficacy studies is .5 to 1 mcg/mL depending upon the temperature of incubation. We henceforth will use the one mcg/mL concentration, the more conservative one.

We also have data in the mouse model. This was a study done by Byrne et al. at USAMRIID, I believe. This was a treatment of experimental pneumonic plague in mice. These mice, too, were exposed to 100 plus or minus 50 LD_{50}s of Y. pestis.

I have displayed the pharmacodynamics data here as well as the survival data here. They gave two different doses in this study, 12 mg/kg every 6 hours for 5 days or 20 mg/kg every 6 hours for 5 days. At early treatment, that is, 24 hours after aerosol infection, 80 percent survival at 12, 80 percent survival at 20. If they treated later 42 hours 32 percent survival at 12, 85 percent survival at 20.

So at this level, if you're looking, there's an increase in survival with dose. If I want to try to look at a pertinent pharmacodynamic parameter T max, or time above MIC Cmax to MIC or AUC to MIC, there really is no change in any of these parameters. That is, the difference in the parameters across doses does not stand out. Whether you select time above or AUC to MIC it all goes in the same direction. You can't really identify a single index that you need to focus on.

Plus we have here a value less than 10 to

1. Yet, at least in early treatment, comparable survival. Some discrimination in survival with late treatment. What this means we really don't know. So we are left with the question of whether or not it's exposure or peak that's important in how we dose gentamicin.

This slide really is a cartoon that portrays the dilemma. With a single dose of 3 mg/kg in a monkey we certainly can achieve peaks to MIC of 10 to 1 or 1 mg/mL MIC. What about this time interval or what about this interval during the dose period where the concentrations fall below the MIC?

The monkey has a much faster clearance than humans, as I indicated. By four to five hours concentrations of 3 mg/kg have really dropped below 1 microgram per mL. You are having a period now with a single dose of about 20 hours with drug below this level. That becomes the issue.

What to do? The first efficacy study then we conducted to this point we decided we would give two doses 3 mg/kg to take care or to address this area where we were concerned about lack of exposure. Then we came back with a second study and gave only a single dose keeping the peak constant but decreasing the exposure by a half.

This slide shows the designs for the two efficacy studies. The first study 16 animals total, 10 of them were treated. The second study 12 animals total, 10 were treated. In the first study, as I said, we gave it twice daily for 10 days. We did some limited sampling on study days 2, 4, 6, 8 and 10.

This sampling was at 5 minutes post-dose, not the peak -- post-infusion, I'm sorry, not the peak necessarily. These were 20 minute infusions so the sample was taken at actually 25 minutes from zero. That is an important point that I'll address a little bit later.

The second study we added some additional sampling that looked at three hours post-infusion so that we could have a look at how the monkeys, now diseased monkeys, versus normal were faring the drug.

We refined our population models. As I said, this is sparse sampling so the only way we can now do the things we want to do down the road is continue to develop our population pharmacokinetics model. We go here from a two compartment to a one compartment model.

Here is the data from these two studies, the pharmacokinetics data. Then I have some survival information, very limited just in terms of the number of survivors. I won't go into detail about the animals that survive versus those that died, what the clinical conditions were and so forth.

These are the observed values from the actual study. These are the calculated values. I did this to show that I'm in the ballpark of my estimated or derived values. The most important values that I want to look at are the Cmax to MIC and the AUC to MIC values, both at twice daily and the once daily dosing. You can see that everything lines up as I had suggested. We keep the peaks constant and we vary the exposure 44 versus 22. Our peaks are about 14 micrograms per mL so well above a 10 to 1 ratio.

Now, how to take this exposure information whether peak or area, and look at human doses that might match up to this. Again, this is a cartoon type of slide just to demonstrate what the dilemma is here. This is a single dose in the monkey. This is a single dose of 5 mg/kg in humans. You can see that the peaks are roughly the same. Exposure in the human is much greater.

If you divide the five mg/kg in half, 2.5 mg/kg every 12 hours, you see that the exposure is probably about the same compared to the monkey. The peak is substantially less in humans than in the monkey at both zero and 12 hours.

So what I wanted to do then was look at human pharmacokinetics in gentamicin and get a feel for what doses might match up. Of course, gentamicin has been around for many, many years. Pharmacokinetics is well studied. These are values in adults. We know clearances. We know volumes of distribution halfwise. We know what key co-variates are in terms of creatinine clearance and body weight.

The 5 mg/kg and divided dosing is approved by the FDA for a variety of infectious diseases but not for plague. Five mg/kg once daily has been used, 7 mg/kg once daily has been used. There are other regimens that are reported in the literature.

What I did was try to identify a study that would give me complete pharmacokinetics information. I did that in this study in the literature and it was done in 939 adult patients. These are the demographics. What I was interested in was the population of pharmacokinetics information so that I could get both intra and inter-subject variability.

I did simulations then of human dosing at various schedules and looked at how they matched the targets that came out of the first efficacy study.

That is, a Cmax MIC of 13.6 and AUC MIC of 43.9. Whether it was 5 mg/kg every 24 hours, 2.5 every 12, or 1.67 every 8 in terms of exposure, 98 percent of the time the human dose will match it.

In terms of peak, however, only the 5 mg/kg matches 73 percent of the time with a range. This was the predicted range in the monkey study, 9 to 19.6. In the humans 20 to 97 percent of the time would be the range we would get Cmax to MIC ratio that match those targets in the monkeys.

To explore further these various doses is it reasonable to go back into another monkey study with another set up doses to determine whether peak or exposure is important, there now is available hollow fibrin methodology. As Dr. Albrecht said, it has been used successfully in looking at levofloxacin and B. anthracis. It also is available now for Y. pestis and it really is an excellent approach to minimize animal exposure and get a lot of information.

In summary then, this is a process that I tried to take you through. To identify animal model that best represents the disease course you need to do the pharmacokinetics, toxicology and toxicokinetics in the animal model of interest.

You need to determine for antimicrobials the appropriate PK/PD index or target based on varying dosing schedules in the animal model, if feasible, but really thinking about using in vitro systems to make it a more efficient process. Population of pharmacokinetics and simulations is valuable in estimating the frequency of achieving these targets with human dosing regimens.

My conclusions are, again, driving home those various points in the process. Lastly, acknowledging the people that I had the pleasure of working with on this project. Thank you.

DR. SANCHEZ: Questions?

PARTICIPANT: Thank you for your presentation. Obviously you and me, at least, we share common sense in terms of PK/PD application in animal model developed from validation which is very important point I brought to the FDA panel during the recent meeting in D.C.

I think everyone here is planning to do animal model developed for animal rule really need to think about the PK/PD similarity between the animal species you are going to use and human if you have data obviously. Probably a lot of times you probably don't have enough information. That factor had to be considered early in that model, especially when you have several potential candidate models to select. That is probably the factor we all should be considering.

A wonderful technique for PK question for gentamicin in monkeys not only near a PK protein, I guess. That's linear.

DR. PELSOR: I'm sorry?

PARTICIPANT: The kinetics in monkey for this drug is linear PK I'm assuming.

DR. PELSOR: We dosed at the lowest dose. If you recall from that single dose study, we looked at 3, 4.5, and 6 mg/kg. There is a hint of nonlinearity in the pharmacokinetics of gentamicin in this monkey model but we did work at the lowest dose level.

PARTICIPANT: Yes. That's what I suspected. It really seems not that straightforward because especially when you look at the Cmax or MIC the low and high dose of the once daily and twice daily that's quite similar. That means probably some absorption problems with the monkey.

DR. PELSOR: I didn't show the diagnostics on any of the population modeling. I agree with you there is a hint of nonlinearity in the single dose kinetics. The behavior, the ability of the model to predict the concentrations was very, very good so I felt comfortable that we had captured the information with our model and that we weren't struggling with nonlinearity in that model.

PARTICIPANT: Thank you.

DR. SANCHEZ: Other questions?

MAJOR ALVES: Once again, I know at USAMRIID there is going to be, or there is another animal model in development for aerosolized plague and that's the cynomolgus macaque and that's going to be put out -- the manuscript is actually in the works with Adamovich and Adamovich and Jolynn Ramon at USAMRIID.

Secondly, now that we do know that aerosolized plague that fibrin deposition not due to DIC but actually due to the agent itself causes or may play a very important role in the pathogenesis of aerosolized plague, have you considered that in any of these studies?

DR. PELSOR: No. That was not incorporated into this analysis, no.

DR. SANCHEZ: Okay. We'll go on break now and return in 30 minutes for the final presentation.

(Whereupon, at 10:17 a.m. off the record until 10:48 a.m.)

DR. SANCHEZ: Okay. Final presentation of the meeting will be given by Barbara Styrt of CDER, regulatory perspectives on use of animal models to study therapeutics for filovirus infections. After the talk we'll proceed to the panel discussion.

DR. STYRT: Good morning. I would like to thank the organizers for their remarkable amount of expert information they pulled together in this meeting. I am going to try to give a little perspective on the use of animal models for therapeutics for filoviruses from the standpoint of antiviral drug review.

I think that following the other talks you've heard this morning that you may consider the status of antiviral product development in this area its rather primitive state relative to vaccines and to other types of drug development could be considered exemplified by the fact that when the organizers were looking for examples of how animal data had been used in therapeutics development they wound up with two examples that are not from antivirals at all and that the speaker who was talking mostly about antiviral product development elected to spend a lot of his talk on vaccines. I think that is just an illustration of how little information there currently is about antivirals in this area.

I want to give a general context of the mechanisms for facilitating therapeutics targeting life-threatening viral infections and encouraging early discussion of development plans with the FDA. I'll talk a little bit about the expanding on what we know about potential uses of animal data and not repeat too much of what you have already heard about the Animal Rule.

Talk some about the special provisions the FDA has for enhanced interactions in these areas. Give a little bit of an idea of the comparative state of the science base and how we are looking at things in anti-viral drugs relative to the review of vaccines and of countermeasures for bacterial biothreats. Briefly touch on some of the highlights of unresolved scientific issues that have already been mentioned and give some specific information about getting interactions started with the FDA.

I should repeat, as others have done, that any opinions I express are my own and that the discussion at this meeting is general informational and does not create FDA policy or provide guidance for any specific development plan.

The sequence of interactions with the FDA.

This is something I want to emphasize and I'm going to come back to a couple of times. In antiviral drug products we put a lot of emphasis on pre-IND consultations in the development of products for agents like filoviruses.

Mark Abdy mentioned a number of ways of starting interactions with the FDA including pre-INDs. We consider pre-INDs to be quite important and this may be partly because of the way that records are kept in different places. These provide a very important way of starting interactions, starting communications. These do not have to be meetings.

You do not need the type B meeting request that somebody mentioned yesterday. These are typically written communications that get written responses and can go through several incremental interactions. These can be requested by government, academic, or industry sponsors. They can include very preliminary data and development questions that can be used for discussions of the development of animal models.

In some instances we have had pre-IND consultation requests when people had not yet decided on the compound they proposed to develop as a drug but did want to talk about approaches to development more generally.

These can go through several increments of interactions before the submission of an IND investigational new drug application which would follow the identification of a compound for development by the sponsor and is needed for the first U.S. human protocol but can include numerous types of both early and advanced studies as development proceeds. Again, animal models and animal studies can be discussed throughout the pre-IND and IND periods of development in parallel with the discussion of human studies.

The overall objective of development obviously is to progress toward an NDA or a BLA for approval or licensure based on adequate and well-controlled studies that support that the product will have the effect it purports to have, and the Animal Rule doesn't remove the requirement for adequate and well-controlled studies but sort of shifts some of the burden to performing adequate and well-controlled studies in suitable animal models.

Again, the development plans generally include consideration of what kind of post-marketing studies might be needed even after an NDA or BLA to confirm effects to monitor safety and so forth. There are a number of mechanisms that I'll get to later for facilitating interactions for a promising drug that may address an unmet medical need. In all of these it's important to look at the risk benefit and the balance of scientific evidence in terms of what measures are appropriate at a given point in development.

Now, just a little bit of organizational information. I come from the Division of Antiviral Products in the Office of Antimicrobial Products in FDA's Center for Drug Evaluation and Research which reviews proposals and data for new antiviral drugs or new uses of existing drugs for viral infections and also reviews drug products proposed as immunomodulators for viral infections.

Since the most recent reorganization also reviews antiviral therapeutic proteins and monoclonal antibodies so we use the biologics regulations as well as the drug regulations.

We do have active collaborations and consultations with other parts of the FDA as appropriate including our colleagues in counterterrorism areas, in diagnostics areas, in vaccines and blood products and, of course, with reviewers dealing with other types of infectious diseases. The evaluation of each development proposal is very much on a case-by-case basis and we want to stress the importance of communicating with the FDA early. We encourage early and frequent communications.

To give a little overview of the potential uses of animal data and, again, some of this will repeat what you have already heard, but I want to make clear that the Animal Rule is not the only way of using animal data and that even when the Animal Rule is under consideration, this does not mean that you don't think about human data and human studies.

In any development plan it is likely that both human and animal data are going to be relevant in varying combinations, and it is important throughout the process to consider the extent of the human data that can appropriately be obtained. It's been pointed out that there will always be a need for safety and PK data in humans. I think it's important to remember that PK with antivirals is not equivalent to immunogenicity with vaccines. They don't really tell you the same thing and that often the kind of supporting data that you need to support extrapolations from PK may be very limited in its availability when you are considering antivirals.

If there is a surrogate marker that can be used for product development, then that should be discussed early on because that could potentially lead to approval under the accelerated approval regulations and Dr. Albrecht gave you some examples of situations in which accelerated approval based on a surrogate pharmacokinetic endpoint was used with supporting data from animal studies so the animal studies were important but were not the pivotal basis for approval.

For human studies it is also important to consider throughout the development process under what circumstances it might be important to have protocols available for use of product in an emergency.

Once there is enough supporting safety and activity data to begin thinking about potential uses of a product in an emergency, discussions should be initiated of what kinds of protocols could be developed so that if a product is used in an emergency setting either with a single patient or in an outbreak setting that you can have interpretable data to the extent possible.

I think that we heard from CDC yesterday about some of the situations in which people have tried to obtain as much information as possible about the effects of interventions in filovirus outbreaks to date including the effects of infection control precautions, and the effects of using blood from survivors to try to treat patients with filovirus infections.

I think this just illustrates the fact that people are trying to get more information about these diseases even though they are hard to study and occur mostly in remote resource-poor situations. If there is enough advanced preparation, it may be possible to use any information obtainable from use of these products in the most constructive way both for the patients at the time and for further drug development.

We would expect that once people have enough information to justify development of such protocols that there would be interest in making the products available in ways that would carry the maximum benefit for the populations most at risk from these diseases.

The use of animal data is not just limited to the Animal Rule but has wide applicability for exploring the antiviral activity of products, the dosing, the effects of different durations of treatment and different timing of initiation of treatment and can provide supporting information.

I think this is something that Dr. Nuzum mentioned yesterday that animal studies actually can provide supporting information to maximize the efficiency of human studies in settings in which human studies can feasibly and ethically be done.

The overall approach to development of these products, the most appropriate question may sometimes not be so much does the Animal Rule apply here as how can the combination of whatever kinds of human and animal studies can appropriately be done. How can these be put together to provide a comfort level that the product will work as intended when it is used in humans? Discussions throughout the pre-IND and IND development periods can help to define these combined uses of data.

I am not going to list again the components of the Animal Rule as you have heard multiple times already, but I will turn them into questions when thinking about use of the Animal Rule that there is always a first question of what kinds of human studies can be done, what kinds of human studies are both feasible and ethical, and can these provide either pivotal or supporting information in the course of drug development. Is there suitable surrogate marker or other mechanism for pursuing approval, in which case, as Dr. Abdy pointed out, the Animal Rule would not apply.

How well understood is the pathophysiology in both animals and humans. This can be of greater concern when you are trying to extrapolate from one virus to another than when you are looking at exactly the same pathogen in humans and in animals. How well characterized are the animal models and what is the evidence that they can be expected to predict human treatment responses.

Do you have relevant endpoints in the studies? Can adequate data be generated to support the dosing that would be used in humans? Dr. Pelsor has given a nice example of the complexity that can sometimes be involved in this discussion.

Again, throughout the process are there adequate plans for performing human studies if appropriate circumstances arise for those studies to be performed? One of the elements of the Animal Rule is that even if a product is approved under the Animal Rule if suitable circumstances arise human studies are then expected to provide -- in order to provide confirmatory information.

Another point in the Animal Rule that I should probably mention is it does say that the agency may use any additional supporting information that is available to it. As you have had illustrated already, the amount of available supporting information can vary tremendously between products and diseases and can affect the discussions of how Animal Rule development might proceed.

There are a number of FDA provisions for trying to enhance interactions in the development of promising products for unmet medical needs with the effort to balance expedited access to products and the scientific integrity of the development process.

I have already mentioned and will emphasize again, we feel that early pre-IND interactions are important for the case-by-case evaluation of the science base and development plans for each disease and each potential product.

Fast track, Dr. Abdy mentioned, is a provision for certain kinds of enhanced interactions and rolling review provisions that can be requested at either early or late development stages. There is guidance on the website that can provide some of the criteria for requesting fast track designation.

Again, at suitable times during the development process it is appropriate to initiate discussion of whether an accelerated approval approach with a surrogate endpoint or an Animal Rule approach may be appropriate. When development has progressed to the point of an IND or BLA whether the product meets criteria for priority review which would accelerate the time clock for review of the marketing submission.

Does this mean that a product cannot be used until all of these stages have been completed? Well, as you know, if an emergency arises when a product has some supporting data but not enough to put together an NDA, there are multiple ways of both making the product available where it may be beneficial and continuing to collect information regarding its potential benefit, and those can include multiple types of protocols under IND, a special kind of IND called a treatment IND. Somebody mentioned this morning emergency use authorization which is a means of providing marketing availability of a product under specific declared emergencies.

For any of these we would suggest that starting out with the pre-IND and IND discussions is the best way to make sure that your position to request one of these facilitated access procedures if appropriate circumstances arise and, again, that throughout the development process there should be a discussion of what kinds of human protocols would be most appropriate to use if a situation arises in which they would be considered important to use.

There are a couple of recent developments with regard to expanded access to products. You may be aware that the FDA published in the Federal Register last December a proposed revision of the regulations governing expanded access to investigational drugs for treatment use that carries some discussion of how the population size that might be involved, the characteristics of the disease, and the risk benefit balance would be considered in allowing expanded access under protocols other than conventional well-controlled development studies and also how the agency would consider granting such access while avoiding or minimizing interference with the clinical trials that are needed for development to actually demonstrate the benefit of the product.

Emergency use authorization. There has been a draft guidance on the FDA website for some time. A final guidance was posted just a couple of months ago, actually late July. This describes the marketing availability of an unapproved product or an unapproved use of an approved product for a life-threatening condition that may be made temporarily available during a declared emergency.

It does not replace the studies that need to be conducted to support approval but does provide another option to be considered for making a drug available if there is some evidence for its benefit but not enough to support a marketing application.

All of these mechanisms of facilitated availability consider both the character of the disease, the availability of other products to treat the disease, and the available information about the risks and benefit of the product. Again, for all of them, starting discussions under the pre-IND and IND mechanisms will make it more possible to be prepared if an appropriate time to use these mechanisms arises.

Just a little bit of comparison of how the science base available for different diseases and for different types of products can affect what additional studies need to be done to advance the development of those products for those diseases.

We sometimes need to look at things rather differently when we are considering antiviral drug development relative to vaccines for either bacterial or viral diseases and relative to some of the products that have been studied for bacterial biothreat agents.

Just as examples in this very short time, when you are looking at antiviral products and comparing their development to vaccine development, some of the things that will need to be considered include the timing of intervention relative to the virus exposure and also sometimes very important the timing of the intervention relative to the onset of viral illness.

How you define viral illness considering how you are going to know a person is sick, how they are going to present for care, what you expect the clinical status of the person with the disease to be at the time that you are able to initiate the treatment relative to what you are able to do in an animal.

And what is the status of the understanding of any markers that might predict clinical benefit? Again, there is not a direct homolog in antivirals to the use of immunogenicity data which can be extremely important in vaccine development. Any markers that are available how much do you understand about how they predict clinical benefit? Then what are the potential targets in or on the pathogen that you may be aiming at with your product and different mechanisms of action of the different products that may be considered.

When we compare study of antivirals for an infection like filovirus infections with some of the study of antibiotics for anthrax and plague that you've heard about, some of the compare and contrast considerations that need to be discussed include how extensive is the understanding of the host pathogen interactions involved in the disease, how extensive is the understanding of the host specificity of the pathogen, the diversity of pathogen species and strains, and the implication of that diversity for the pathogenesis of the disease.

What is the extent and understanding of prior human experience with the drug both with regard to the safety database and to any information about efficacy in similar diseases or other diseases against which the product may have activity. And what is the extent of understanding of pharmacokinetic, pharmacodynamic parameters and their relationship to clinical outcomes.

For some brief examples, and most of these have already been mentioned in the last couple of days, with filoviruses the differences in pathogenesis of different filovirus species and strains and different hosts are one of the examples of areas that are not completely understood at this time.

Other examples of unresolved scientific issues, the relative importance of viral replication and host responses at different stages of the infection and the illness, particularly important if you are thinking about something that may be used relatively late in an illness. The relative balance of beneficial and deleterious components of host responses at different stages of infections and illness.

Again, very important when dealing with proposals for products that are intended to target elements of the host response during treatment of an established illness where as it's been pointed out several times something like modifying the inflammatory cascade might be beneficial at one stage of the disease and might be not just not beneficial but actually harmful at another stage of the disease, how much is understood about that.

And the implications for antiviral interventions of all of these. The same intervention might have very different effects in different clinical settings. Correlation is not identical to causality. The fact that two phenomena are observed together during the natural disease does not necessarily mean that changing one of those is going to change the other one or is going to change the outcome in the way that is hoped.

Then the potential impacts of the magnitude of intervention. Some kinds of host response modifiers could have paradoxical dose responses, potential impact of the timing and the duration of the intervention. I'm sure that as we learn more about the filoviruses we will start to recognize some other unresolved scientific issues.

We all have advocated repeatedly communicating with the FDA early and often when considering development of animal models and considering development of products to address these infections. As I have mentioned, in antivirals the pre-IND consultation is generally the most efficient and effective mechanism for initiating the interactions about specific aspects of development.

The request for pre-IND consultation can take place very early in the development process. The sponsor can present to the agency their initial data, hypotheses, proposals and questions often for written feedback, and this can be incremental. The first request for feedback will not answer all the questions.

It may lead to some suggestions about

additional information that is needed in order to address some of the questions, but it is a way of getting the interactions started and helping with the focus of the information generation. This can provide a venue for discussion of the plans for animal studies.

Both development of animal models and uses of animal models and the content of an eventual IND submission, can identify characteristics of the products that may affect the study plans including toxicity, route of administration and delivery to the relevant anatomic sites can be very important in some of the antiviral discussions, mechanisms of action of the product and can begin an incremental dialogue to continue throughout the pre-IND and IND development processes.

As I mentioned, there are a number of guidances on the FDA website that provide additional information about some of the topics we've been referring to. There is a website where you can get more information and contact information about the pre-IND process. I found from my own attempts to search the FDA website that the most efficient way of getting to that site is by going to the FDA CDER, that's Center for Drug Evaluation and Research, website and typing pre-IND into the search box.

That usually gets you to the pre-IND information site fairly rapidly. I would also encourage people, even if they don't think they have all the information that's listed on the pre-IND website, much of which is sort of suggestions about how to optimize a submission, to go ahead and contact the appropriate review division so that you can start interactions because an initial submission can be taken in very preliminary form for feedback.

Questions?

DR. SANCHEZ: Thank you, Barbara, for that very informative presentation. Questions, please?

PARTICIPANT: Thanks for a good presentation. I have a couple of comments about the antiviral drugs and anti-filovirus drugs. It's kind of curious for us. We are in the opposite situation for anti-filovirus drugs as compared to antiviral drug development in general.

In general the animal models for viral infections, human viral infections, are quite poor. If you look at the regulatory process for anti-HIV, anti-hepatitis C, you name your virus, animals are not a big component. Animal efficacy is not a big component. We don't even have animal models for hepatitis C.

This is sort of the flip side of the coin this Animal Rule for filoviruses where we can't test it in humans and we are looking for the animal model. From that perspective the animal models that we have, mouse, guinea pig, nonhuman primate, are fantastic compared to the other fields.

The second comment is a lot of the discussion today has been about approved drugs being redirected to another purpose. We don't have that situation in terms of anti-filovirus drugs either. There are some efforts to do that but we are generally talking about unknown against unknown here. A lot of these issues are going to be much, much more complicated.

The real issues come down to the preclinical evaluation when you are doing the efficacy. How do you get into the animal model? What kind of work do you need to do to justify putting it into these animal models because it hasn't been pointed out but in a vaccine efficacy model you vaccinate however many times and then you challenge one time.

In the therapeutic model, as was mentioned in one study today, you have to treat multiple times hopefully based upon the PK. You're talking about five to 10 animals, dosing three times a day. You may be testing multiple drugs over multiple days. You're talking about exposing people in the BL4 to infected animals quite significantly. These issues are so much more complicated and need more time to be discussed than has been discussed at this meeting I think.

DR. STYRT: I thank you for neatly encapsulating some of the ways in which this area is so challenging and the fact that, yes, there has not been a good track record with using animal models to see how you can predict human outcomes with antivirals.

To the extent that it's been tried it hasn't worked terribly well. The animal studies that may need to be considered in these areas can be very challenging and pose their own risks. I absolutely agree with you that these are issues that need a great deal more discussion.

DR. SANCHEZ: Questions?

PARTICIPANT: On one of your slides you mentioned treatment IND. I don't think many people know so is that authorized only on EUA or what is the difference between a treatment IND and a regular IND? Can you just give one of the examples?

DR. STYRT: Just very briefly, especially because as Mark Abdy mentioned yesterday, he didn't talk a lot about late development because filoviruses have a long way to go. We haven't heard about anything that looks like it's quite ready for this yet. Treatment INDs are protocols that allow the use of a product for treatment usually after there is a fair amount of information available about risks and benefits, safety and at least preliminary efficacy information.

Perhaps the best example of how treatment INDs have been used traditionally is that when products have already gone through most of their clinical trial history so that they are getting somewhere close to being ready to be reviewed for approval but they are not quite there yet.

Patients who are not eligible for or do not have access to a clinical trial might be treated under a treatment IND protocol that might allow some additional observational data and safety data to be collected but would not meet the standards for an adequate and well-controlled trial.

That is something that is usually considered when a product is far enough along that you know a fair amount about how it works in people. An emergency use authorization is a specific way of allowing use of a product under emergency circumstances in a setting that is not a clinical trial. A treatment IND is still a clinical trial. It still has informed consent provisions.

Emergency use authorization is not a clinical trial and does not have informed consent provisions but does have requirements for making information available both to physicians and to patients and does have, if you look at the guidance that was just recently posted on the website, some provisions for at least discussing what additional information can be obtained about the product.

These are both measures that might be used when there is some information available about risks and benefits of the drug. It is not ready for a full NDA, but there are reasons to consider using it. I cannot give a specific comparison because that's going to depend on the setting.

They have some important differences but there could be situations in which it would be appropriate to think about both of them and to enter into discussions about both of them to be prepared for one or the other to be used if needed.

DR. SANCHEZ: Any other questions? I

thank the speaker for a very interesting session, and I guess we move on to the panel discussion.

DR. JOHNSON: Okay. I think we will go ahead and get started. We will need to end promptly at 12:30 so that some of the panel members and members from the audience can be sure to meet their travel arrangements and not be delayed at our airports for a night.

My name is Robert Johnson. It is my pleasure to serve as the moderator for this panel. I'll kind of caveat that I am not a filovirus expert. My job here is more to make sure that the discussion flows well and let the experts do the talking.

The way we are going to set up the talk for this afternoon, since we only have an hour -- just a little over an hour, I want to be sure that we have a chance to have all of our panel members comment on the questions so we are just going to go through the list of fairly broad questions one at a time, and we are going to let each of the panel members provide any discussions or thoughts.

We'll use that as a starting point for going through each of the questions. Then at the end of the question after we have had some discussion I'll try to summarize if there is any consensus. If there isn't, that's just fine, and then we'll go ahead to the next one.

I would like to try to get through as many of the questions as we can. I have a feeling the first couple questions will take up a fair amount of time. I think with that we might as well go ahead and get started with the first question. I'll note these questions were set up so they are pretty broad. The purpose here is really to kind of get some general thoughts.

We are not addressing any particular one point so it's a chance for the panel to kind of give their general thoughts of some of the pluses and minuses of the different animal models and where maybe there are some holes that we can fill or where maybe the animal models -- where there are some things lacking that we are just going to have to learn to deal with.

We'll start with the first question. What are the similarities or differences in considering developing appropriate animal models for therapeutic counter measures as compared to that for the vaccines?

Maybe, Mike, I'll ask you to start if you just have any thoughts on that.

DR. BRAY: Okay. I actually brought up

this question very briefly yesterday afternoon during the other panel discussion when we were talking about what do you need to know about the animal model that you are using. At that time the topic was vaccines.

I suggested that actually there was a difference between the amount of knowledge you needed for vaccine development or the type of knowledge, that this could be different from developing therapeutics. Specifically if you are trying to develop drugs, something you are going to use after an animal is already infected and possibly after the animal is already ill, clearly you need to understand everything you can about the disease itself.

With vaccines you are looking at -- you really want to understand immune responses. Ideally a vaccine that is given pre-exposure will prevent the disease altogether so the details of the illness itself aren't quite as important.

I don't know to what extent you need to know -- you clearly need to have some understanding of the disease but if a vaccine works very well, it lessons the requirement of how much you need to know about the whole disease course.

I think when we are talking about therapeutics in the filovirus field we need to make the distinction between drugs that directly target viral replication, that target the polymerase, that target viral transcripts to try to knock out messenger RNA.

Distinguish between those and then others that target host responses. In the second case, again, you need to know much more about the disease and how the host responds to the infection than you would in the first place.

DR. SANCHEZ: In the therapeutics I think that with antiviral drugs the animal models may not be as important as in vaccine development. Especially if the drug is targeting a common compound in the cell that shows very high conservation. Then you can perhaps rely on results from an animal such as a mouse and compare that upwards into nonhuman primates.

DR. REED: I would tend to think that in the animal model here you need to know a lot more about the disease and in particular biomarkers that are going to determine when do you treat. When are you going to see a patient in the hospital setting and how does that apply to your animal model? Cytokine levels, in terms of the immune response, are going to be just as important here I think in terms of the innate immune response perhaps more than the adaptive.

DR. BAVARI: So I go back to the exposures that have already occurred. During 9/11 I don't think everybody waited to see, for example, to get CFUs before they actually start treating people. I think they just initiated the treatment even before they knew there was a perceived implication of being exposed. That, I think, opens the door even for pre-exposure therapeutics. I know everybody likes the discount, but I think it's as valuable as it is therapeutically coming back.

I think in the case of filoviruses if you are coming back to therapeutic -- maybe I'm going into one question from the other -- if you wait you have viral titers that are really detectable by pfu. By the time you get to pfu and you get your QRTPCR data back, you are already talking probably about four or five logs.

I think at that point there's not much you can do. I think you have to have better ways of monitoring. You said biomarkers. I think that's great. I think that anything we can monitor the exposure and get to them as fast as possible diagnosis and diagnostic I think probably comes to mind. Go ahead.

DR. SANCHEZ: I also think the situation

if it's an outbreak situation, that's going to be a whole lot different than one gauging a whole lot of symptoms and saying, "This looks like it might be Ebola and we need to do something." During an outbreak one would also have case definition of the things that one might be able to apply instead of going through extensive testing that might delay the treatment. It depends on a lot of factors, I think, in how one proceeds.

DR. JOHNSON: To kind of get back, I think, maybe if we could for a minute to some of the comments about understanding the animal model itself and some differences between the vaccines and the small molecules. I guess earlier we saw in the talks some of the things that were considered for other products both by the developers and the agency in looking at things. Some of the important things were along the lines of the pathogenesis and the disease course.

I guess maybe what I would like to ask the panel is what are your thoughts in terms of -- maybe we can start with the mouse model, frankly. How does the pathogenesis and the disease course of the mouse infection model compare, say, to the human infection model or even to the nonhuman primate model?

DR. BAVARI: I think the mouse model is actually very, very similar to nonhuman primate model. The differences are small. Might be significant but the differences are small. Either we'd have to rate it -- it's not something that you can jump over. To do large scale type of therapeutics there is no way that you can take everything into nonhuman primate.

There's got to be a path and that path is through murine models and then into nonhuman primates. The way, at least we've been working, is that we understand the differences. Mice are not nonhuman primates, I think we all recognize that, but they are not mosquitos. I think that really needs to be driven home that it does have a place in it. There is no other way to dissect the pathogenesis except by really going through these models.

I think for a lot of screening mice is a great place to start. If they don't work at that point, I don't think there is a reason to continue. If they do work, then you want to go to guinea pig.

DR. REED: You should also include the guinea pigs in there. Guinea pigs do tend to have -- one of the things we've seen is we've done some very limited studies with mice. There are some issues there with the route of infection. Guinea pigs are susceptible to aerosol. At least we've done yet -- so far with mice we don't but there's issues there with the concentration of the virus and the dose that we can get in the animals that we need to overcome first.

DR. BAVARI: Sorry. Repeatedly we have seen D-dimers going up even in mice and in guineau pigs so that is nonexistent there. We've seen very, very similarities between murine model and nonhuman primate models. I think they are very closely related and they shouldn't be discounted.

DR. BRAY: I think some of the differences that were between the mouse model and nonhuman primates that were thought to exist 10 years ago may not be as true nowadays. Sina was just alluding to coagulation studies. When I was trying to do those in the late '90s at USAMRIID were nowhere near as advanced as they are now. There are good data now showing that D-dimers can be detected in mice and can be measured and may prove to be a very valuable biomarker.

Another consideration here is that even though rodents and nonhuman primates are clearly different, one of the advantages of working with mice is that you have the tools and the reagents available to actually figure out what those differences are.

Yesterday Mike Taka was simply referring to the fact that mice apparently have much stronger or more effective type 1 interferon responses to wild type filoviruses, but that's something that can be eliminated in mice either using knockout animals or using antibody to type 1 interferon, so you could potentially correct for this and actually try to come up with a modification as sort of a further test of a drug. Does it work in an interferon deficient mouse, for example, which at present with guinea pigs the genetically modified animals simply aren't available.

DR. SANCHEZ: But we now know there are differences in immune responses within human adaptive responses. You take a look at the difference in the immunology from the mouse and the human, it's very hard to get around that.

DR. REED: One other thing you are going to have to factor in, and this just occurred to me, is one of the issues I've seen with the animal models if you look at the human data, the onset of clinical signs to death is considerably longer in a human than it is even in a nonhuman primate.

It's not the 48 hours or so that we see in a nonhuman primate. It's typically much longer so that has to be factored in, too. If you could come up with an animal model with a longer disease course, that might be better suited for a therapeutic study and provide you better data.

DR. BRAY: Actually, in terms of the difference between vaccine development and therapeutic development, probably a fairly important example here is the models that we've heard about, the nonhuman primate models, have been uniformly lethal models, the Marburg model that Tom Geisbert talked about and the fact that people tend to do all their work right now with the ebola Zaire virus.

Tony is quite aware that for ebola Sudan the survival rate in humans is roughly 50 percent and has been that repeatedly in a number of outbreaks. We don't know anything from nonhuman primates about how humans manage to survive that infection.

If you are trying to come up with a vaccine against Sudan, probably the same platforms that work against Zaire would work against Sudan, but if you are coming up with a treatment for Sudan, you would really like to know how humans managed to survive and how can we model that in nonhuman primates. Right now we don't have that model.

DR. SANCHEZ: There's indications that the MHC profile HLA-B is important in that. That's one thing with the monkeys is we don't have a clear insight as to their profiles or what are important. Tom Geisbert talked about differences in African greens, cynos, and rhesus. I think that is something that very much needs to be explored.

DR. BAVARI: Maybe we haven't really spent a lot of time talking about guinea pig model but, as Doug actually pointed out, it's a valuable model. Based on at least our experience, every time that we've gone into guinea pig and we've seen data, we could literally reproduce it in nonhuman primates so I think it's at least for therapeutics.

The vaccines there are some other issues maybe but at least for therapeutics if you see 80 or 90 percent protection, that gives us really a bar, and you're a lot more satisfied then to walk into nonhuman primates than to go directly from the mice into nonhuman primates. That bridges really nicely for us.

DR. JOHNSON: So do you see any difference in the predictive effect of the guinea pig model if your therapy is against the virus versus a host cell response?

DR. BAVARI: That becomes other issues that is probably a lot harder to discuss. There isn't really enough information available. There is not enough reagents available in guinea pigs to really dissect that. That really needs to be dissected at the mouse level which you get chopped down by everybody because it's a mouse. We recognize that. However, we need to start some place and then build it up.

It's a tough question, but viral titer is a clear indication of what is going on. If you can knock down the viral titer, most likely your therapeutics will be successful. How successful it is I can't tell you 100 percent successful or 50 percent As you saw, some of the data that we showed we have in some cases absolutely no viral titer in the sera that we can detect and nonhuman primates die seven or eight days after that.

That's maybe because they are missing other components of therapeutics that needs to be added so how do you do that? Can you do every one of those in nonhuman primates? I don't know if there is enough rhesus macaques out there for us to really be able to do that. I think a lot of that needs to be addressed in a guinea pig.

DR. JOHNSON: I think just one last question I wanted to pose, I guess subquestion in terms of the pathogenesis for the animals that died, the mouse and guinea pig. How does that compare with what you see in humans? That was discussed a little bit yesterday.

DR. BRAY: In my talk I mentioned that although we are talking about filovirus hemorrhagic fever that hemorrhage really isn't an important part of the cause of death for human infections. It seems to be increased vascular permeability and fluid shifts out of plasma.

Just a loss of intervascular volume, failure of organ profusion, shock. Presumably these changes occur in rodents. They certainly have similar cytokine responses that should produce those effects. I'm not sure to what extent they've been measured but I think the actual pathophysiology of death is quite similar.

DR. WARFIELD: Can I just make a comment about the rodent models? There's been some discussion that we have developed some new Marburg mouse models. Actually, Tony Alves here has read a lot of the pathology, and he can provide some of the backup data. I think what we found for multiple isolates of Marburg that we have adapted to mice that have very similar to the ebola virus only a very few nucleotide changes from the wild type virus that we started out with. These viruses have very similar pathology to what we are finding in both guinea pigs and nonhuman primates. Very profound liver changes, lymphocyte apoptosis. The cytokine profiles of the mice are very similar to what we see in infected nonhuman primates, elevated D-dimers, lost of platelets. We are still working on characterizing these, and there is really not human data out there to correlate what we found in the animal models yet, especially for Marburg.

Tony showed some of the ebola data. I think what we are finding with more and more characterization of the immune response in the pathology is that the rodent models are actually very similar with some of the caveats, especially that Tom Geisbert talked about yesterday with some of the coagulopathy. Some of the same biomarkers are still there and I think they are very useful for screening.

Like Sina has said, we've done large sets of antiviral screening in rodents that just really would not have been possible to do in nonhuman primates and so I think there is going to be mounting evidence from people like Doug and ourselves that have really worked very hard on characterizing the rodent models to show that the pathology is very similar.

DR. JOHNSON: Great. Thank you very much.

PARTICIPANT: May I add one more comment regarding the two types of models. When we are testing vaccines as opposed to countermeasures such as suppression of virus titers by more simple ways such as siRNA or antisense, etc., vaccination is a complex interaction of a virus with a host immune system, and filoviruses, as we know, have two type 1 interferon antagonists which are VP35 and VP24.

Suppression of these type 1 interferons is highly connected to development of adaptive immune response and specificity of viral suppressors of type 1 interferons is unclear. There are examples when suppressors of proteins antagonist of type 1 interferon response of human viruses do work in mice and rodents, and there are other examples in which they do not work in rodents.

This is connected and there is a lot of crosstalk between innate response and adaptive response. For these reasons these things explain why rodent model is not highly predictable for filovirus vaccine and there are many examples of other viral vaccines in contrast for the more simple countermeasures such as simple reduction of viral titers by siRNA, for example. I think this rodent model would be highly predictable and even probably -- cell culture would be highly valuable -- would provide highly valuable predictive data for human.

DR. JOHNSON: Great. Thank you.

PARTICIPANT: I don't think anybody here actually disagrees with that.

DR. JOHNSON: I think maybe we'll need to wrap -- I'm sorry.

DR. GEISBERT: No, just a couple comments. I don't want this to come across as anti-rodent because I know I come across that way a lot. I think that rodents do have some utility but I think we are going a little bit overboard here in making some broad generalizations.

The whole issue, and Lisa can talk to this if she wants to with D-dimers, we've looked at D-dimers, or Lisa has, with ebola in the mouse model and really haven't seen much. I think the bigger issue is the fibrin, and it's just not there in the rodent models. It just doesn't happen. If you are looking at certain drugs like NAPc2 or activated protein C which have good activity in nonhuman primates, I don't know how you would do that or evaluate that in a rodent model.

I think there is a lot of differences.

The viremia that Mike published in rodents goes up to 10^8 or 10^9. We know in nonhuman primates it's more in the 6.5 to 7 log range. From what we know about humans the limited data that there is out there, and it's very difficult. A lot of times placque from human isolates, as Tom and Tony will attest, it looks like about 6.5. So I think there are some very significant differences.

I also want to point out the lymphocyte apoptosis, which is a huge factor in human and nonhuman primate disease, yes, it's true that in our lab we looked at that and Stephen Bradstreet showed that there is what we call classic apoptosis by morphology in the mouse model. That is not exactly true.

Yes, there's classic apoptosis if you can find it but it's what we term program cell death like apoptosis. There's different pathways of apoptosis and it's very different in the mouse versus the nonhuman primates and the human. I think we need to be really, really careful. I agree with what Sina said. I mean, you're not going to screen siRNAs or antisense -- or things like that in large numbers in nonhuman primates, but I think we have to be extremely careful.

Mike, I want to answer your question real quick about Sudan. We do see differences with Sudan. We put the Gulu isolate into cynos and we only get about 50 percent mortality. It's only with Boniface that we do better than that.

One quick point with Doug's thing. With human versus nonhuman primate the disease course is going to depend on the dose and the route and a whole lot of other variables, and we've shown that with needle sticks. I think if you look at the rhesus macaque model and you look at some of the oral conjunctival or other lower exposures or Doug's aerosol where you had one survive at 8 pfu, you can really walk that rhesus model out if you lower the dose or change the routes. So all things to keep in mind.

DR. SANCHEZ: Tom, with that fibrin deposition would that account for the very rubbery consistency of the monkey spleens at the time of death because you don't see that in the guinea pig model?

DR. GEISBERT: You don't see it in the guinea pig or the mouse. I don't care if it's ebola, Sudan or ebola Zaire, go look at the red pulp or the marginal zone from a monkey that died. You can't even look at the tissue architecture. It's just fibrin everywhere. D-dimer fibrin degradation products, to use a more broad term, can have a lot of effects. It can activate the endothelium.

There's all kinds of things, let alone just plugging up vessels and hypotension and all kinds of things like that. Because that doesn't happen in a rodent to try to compare a primate to a rodent with that part of the disease pathogenesis is, just to me, I just can't --

DR. BAVARI: I think if you're looking at anti-fibrin type of therapeutics, I totally agree. It's difficult to look at those in rodents. You've got to go to nonhuman primate. If you are looking at things that are directly against the virus, there is really no reason to start with the nonhuman primate. You start with rodents.

And if you want to compare things such as the pfus that Mike generated 10 years ago versus the pfus that you're getting now, they all need to be done side-by-side in the same study to see if you get actually 10^8 or 10^9 viral titer. I understand what you're saying, but we all have -- the way we are all doing the pfus these days are different so they all need to be done simultaneously.

DR. JOHNSON: I'm sorry. I'm sorry, we

are --

DR. GEISBERT: There's no argument about that. It's just like --

DR. JOHNSON: We are running a little short on time here so obviously, as I think we all know, there are several unknowns still within the filovirus animal model field. I think what we've heard today is that there are some similarities amongst the different animal models and that in some situations the guinea pig and the mouse model are predictive of what we see in primates and what we may see in humans.

Clearly there are instances where either for the workers needed or they may not be an appropriate small animal models. I think in terms of overall the animal models one of the things that I heard that is probably going to be an issue that will be important to be addressed down the road is that the time to death is quicker in primates or even your animal models versus what you see in humans.

Of course, as we'll touch on a little bit later in some of the other questions, I think it's fair to say from a therapeutic standpoint we are thinking about treatment after disease symptoms or after exposure. That's an issue that could be difficult to deal with.

I think with that, if it's all right with the panel, we'll go on to the second question which is what initial clinical symptoms should be focused on to identify potential relevant ranges and triggers for timing of treatment initiation for development of therapeutic animal models.

Tony, I didn't know if maybe you wanted to take a first crack.

DR. SANCHEZ: Maybe it depends. In animal models one has pretty much laid out what one can or can't look for in the laboratory. For my part, one can observe them just see outwardly if they are looking bad. But a blood test and looking quickly at liver enzymes, one can easily tell when they are sick and relate that to perhaps a human situation. One might be able to use fever in a situation where the person who meets the case definition there's an outbreak and then one can proceed.

DR. BAVARI: What would you do if there is no epidemiology data or surrounding data that actually indicates that there is a case going on because then you have to have confirmation?

DR. SANCHEZ: Then you would have to have diagnostic confirmation to tell you that it is ebola or Marburg.

DR. BRAY: I think you may need to qualify the question a little bit. If you're in an outbreak situation where you know that you are dealing with ebola or Marburg and you know that somebody has been in contact with a patient and is at risk, then it might be very simple, you know, a person is simply not feeling well would be enough to initiate treatment.

DR. SANCHEZ: Exactly.

DR. BRAY: If you are just dealing with someone shows up in an emergency room and is not feeling well, it's very unlikely you are going to start treatment for ebola hemorrhagic fever. Some of this is situational. What is it that triggers starting therapy?

In general, I mean, people are becoming ill because of cytokines that are circulating. They have fever, headache, malaise. Everything is because of the cytokine response so it may be good if you're going into animals to look at markers. Or, as Thomas pointed out, D-dimers are very early. It depends on whether we're talking about really restricting ourselves to symptoms or whether we have a lab test available.

DR. BAVARI: Would you really wait to get some lab data back? By the time you get lab data back it's going to be late.

DR. REED: You make the point and it's a good one. If you have confirmation that there's an outbreak going on and someone comes in and --

DR. BAVARI: They need to get treated.

DR. REED: Yes. If they meet the case definition, whatever that may be. That's what you've got to set is some kind of criteria for when do you initiate treatment.

DR. BAVARI: I don't know about Tony, but if I have something in my pocket that says only use it after you get confirmation by QRTPCR, I'm going to be injecting it myself.

DR. SANCHEZ: A nightmare situation would be if you had an ebola outbreak in the middle of flu season while there's a lot of people with flu infections out there muddling it all up, and you have a limited amount of treatment available.

Then you would have to resort to sorting out who is most likely to be infected and what case definition if they are linked to other patients might be good and then move on to testing. That's where we are really lagging is some rapid pregnancy type of test where one can quickly do a finger prick test to see if they are acute.

DR. JOHNSON: Let me try to put a little framework around this. Maybe we can look at it in terms of the animal models. If you are working your lab and you've infected your animals, at what point do you start to see some clinical symptoms of disease and what are some of the initial symptoms that you can observe?

DR. BAVARI: Really not much is going on in the first two days so it's very difficult. Maybe by the middle or end of the second day you can start seeing some markers that might be valuable. Before that I think it's difficult to see. I'm worried that if you set up the therapeutic level of protection being two days after you confirm, I think many of the therapeutics will not go there. I just think we're going to have an empty suitcase with nothing in it. We need to be careful of how high we are trying to set that bar.

DR. REED: What we've seen in looking at the primates, in particular going into the rooms and observing the animals, the clinical signs, the first indications to the animal care techs or the other people walking in the room that the animals are sick actually occurs after we see fever start to come up in our telemetry, and that's going to be two days or more after you see elevations in the levels of D-dimers.

I would say if you have a person come in the hospital and they've got elevated D-dimers, there might be something to be concerned about. You've still got that issue of juxtaposing. How do you know it's an infection with ebola or Marburg, and how do you know how to treat then? What if it is flu?

DR. BAVARI: It could be hepatitis. It could be all sorts of stuff so that becomes a problem. Going back to what Tony said, a dip stick would be the best way to do it so maybe you guys at the funding agency you want to start thinking about that.

DR. SANCHEZ: I think the reagents are out there to try to develop these things. It's just a matter of a will to do it. From my experience in looking at the animals systems from the guinea pig comparing that to nonhuman primates, for me there has been no surprises in terms of identifying guinea pigs that look like they are going to die.

They show weight loss, and it's very predictable. Whereas, in the monkeys they can appear very normal up to day four and then all of a sudden one will just die. They don't look bad at all, and then all of a sudden next day they are on the bottom of the cage stiff as a board. I've seen more of those.

DR. REED: And that's what we've seen in guinea pigs, too. You see a progressive weight loss almost from the time of infection, and temperature comes up later.

DR. BAVARI: How is that compared to what you've been seeing in the field?

DR. SANCHEZ: In the field it's very hard to follow the patients. Usually they will come in when they are already acute so you've got a set of symptoms that you'll say, "Well, you look like and we'll admit you and then follow up with the testing, diagnostic testing, and then either pull you out of the ward or leave you there." It's a completely different situation.

DR. BAVARI: I'm sorry. So what do you do, you throw away the nonhuman primate model now because there are some differences between the two, between human and nonhuman primate?

DR. SANCHEZ: No.

DR. BAVARI: That's what I thought.

DR. SANCHEZ: No, it's a good model.

MAJOR ALVES: Just real briefly, I'm speaking as a veterinarian first and then a pathologist second. I think sometimes in a research environment we may as researchers should probably be a little bit more forward thinking and treat it as if some of these animals are in a true hospital setting.

With that we go to the telemetry devices. I had alluded to this earlier that a lot of times with these telemetry devices we can not only just get if the animal's spiking a fever but now some of the telemetry devices actively measure heart pressures, heart rates, and everything else.

I think maybe we need to be a little bit more forward thinking. I know Katie Daddario is looking at doing like blood gases and everything else and those are clinical parameters that are used in the human side of the house and they should be used --

DR. BAVARI: But they are nonspecific, Tony.

MAJOR ALVES: Right, they are nonspecific but --

DR. BAVARI: Every one of them are nonspecific.

MAJOR ALVES: The question is when should we start looking at treating. I think if we have some data on whether these things, blood pressure starts to drop by this amount, heart rate goes up this amount, then I think incorporating everything together that that may provide a timing for treatment.

DR. REED: We have actually looked at heart rate and blood pressure with filovirus infection. We have seen it with plague as well and alphaviruses. What we typically see with heart rate is that it increases after you get your fever and if you look at the ECG pattern it's classical sinus tachycardia. Blood pressure doesn't really change that significantly compared to our base line. We do see with filovirus infection that right before death there is a sudden crash. At that point it's far too late for the animal.

DR. BAVARI: I think there are probably other biomarkers that need to be looked at that may generalize for family of the viruses and maybe specific biomarkers that can be targeted to only go after filoviruses. Now then it's going to become which one and so on but at least you can narrow it down.

PARTICIPANT: In terms of these issues of what animal model and timing of treatment, etc., it really is important to distinguish what we're talking about when we make these discussions. We have already mentioned that it's really important to talk about whether you're hitting a virulence factor or this is a true antiviral meaning inhibits viral replication. That sets the stage for how the discussion -- what model and how to treat. That would be one comment.

Second is where are you in the process. I think at the stage of screening versus the stage of using the model in a regulatory fashion to get approval for the drugs are very different issues. The stage of screening has been brought up. The mouse is a very good model, as is probably the guinea pig, for replication inhibitor because there is lots of data out there that says there is a pretty good correlation if you get the drug in soon enough that you block viral replication and you ameliorate the disease.

Viral load is a good surrogate marker for disease. I think we should operate under that assumption and keep that straightforward. The other assumption I would make is that as far as screening for true antivirals that a prophylactic model is where to start because if you have an antiviral and it can't work in a prophylactic manner, it isn't going to work therapeutically so test it prophylactically. If it works, go there.

The second issue of the animal models is PK. Mice are irrelevant as far as PK so we know that the regulatory model, the model that is going to be presented to the FDA, is going to have to be the nonhuman primate because of the PK issues. Whatever you show in a mouse you are not going to be able to extrapolate that to people from a PK point of view.

Then that begets this whole issue of what are the right PK parameters. We have heard before about Gentamicin and area under the curve versus MIC, etc. What is the right parameter for an antiviral? Area under the curve over what? EC50? EC90? Log reduction? What assay? What cells? It's a nightmare.

DR. BAVARI: To even complicate that a little bit more, the PK data that you get from sera or from urine doesn't necessarily mean tissue level. That's what I deal with constantly is actually the tissue level.

PARTICIPANT: That's an issue that I would ask the filovirus experts about in terms of what is the relevant tissue beyond serum as far as that goes. But my point is that this will have to be done in the nonhuman primate. That's a given but more focus needs to be addressed on what is the viral parameter that we should be looking at because it's really not very clear right now.

If you are doing a study and you get negative results, the two possibilities are that the drug isn't good or it wasn't administered properly and we need better parameters to see how to better administer it. We can't do that unless we have agreement on what the right parameters are.

PARTICIPANT: Sorry. I've got a very quick question. Just in terms of for approval purposes what would you consider to be the appropriate endpoint? Is it survival? Reduction in viral titers?

DR. JOHNSON: That is probably something that you should ask the agency. Really the purpose here for this talk is to kind of get an idea of where we stand on the animal models. We understand the purpose here is that we're not going to answer everybody's questions and we are not going to be able to deal with all the issues, but I think, you know, really the point here is to kind of get an idea of what we have in terms of the general animal models.

Like I said earlier, see where the holes are and see what will work and see what won't. The previous comments were good. We understand that but, again, I think that what we are looking at here is a little higher level than that. I mean, we understand that down the road there are going to be a lot of issues.

The problem we have seen continuously over the last several years is everybody kind of wanting to jump ahead to this thing of, you know, well, you have a model that kills your primates and now we are ready to go for licensure and there's a lot of unknowns.

Kind of the purpose here is just even at the research stage every company, every agency is going to be using their own screening model. You've heard some of the comments today about what different people use for screening and we are kind of talking about some of the good things and the bad things. What individuals choose to take forward, I think, will be their decision.

From a broad sense I think it's fair to say some of the things I've heard today is that with the primates the first symptom you tend to see is fever, and it comes up about 48 hours post-infection. I think that's a good generalization. It seems to be pretty good. With the guinea pigs the first thing you tend to see is weight loss, and that comes up somewhat relatively rapidly after infection. Is that correct?

DR. REED: Fever is about four days after infection.

DR. JOHNSON: For the primates?

DR. REED: Right. I think the D-dimers is the first thing that anybody's seen. It's just trying to match that up because the animal doesn't physically appear sick at that point.

DR. JOHNSON: In terms of looking at, I guess, where the primates are dying versus where the first clinical symptom you see fever comes up there is not much time there as I think the panel also indicated. Maybe we don't really have a great early marker at this point, perhaps the D-dimers. But something, I think it's fair to say needs to be explored more. Is that a fair -- do people agree with that?

PARTICIPANT: Correct.

DR. BAVARI: So really to understand the pathogenesis it still goes back to that because understanding the pathogenesis will actually lead into these type of biomarkers. I think that is critical to actually continue pursuing the interaction, the host pathogen interaction for filoviruses.

DR. REED: And I will say, too, the issue of endpoint isn't necessarily irrelevant here. There is an issue of you look at drugs that affect fibrin deposition, and those affect one portion of the disease.

If you look at other drugs that treat other parts of the disease, you may be talking about some kind of combination therapy that is really going to ultimately be successful. There's going to be an issue of what is your endpoint for your preliminary studies even though understanding ultimately when you get to your pivotal studies the desired clinical benefit in humans is going to be your driving factor.

DR. JOHNSON: So what does the panel think in terms of their general thoughts on what an endpoint should be from a screening standpoint?

DR. REED: I think it depends on the drug and what you're looking at and initial screening. If it's reduction of viral titer because you're looking at viral replication, that might be enough to continue additional studies.

DR. BRAY: I think one of the advantages of working with rodents and some of the data that, for instance, Sina showed this morning on dose response is that you can do experiments that are large enough and you can use inbred animals that are quite consistent and you can actually measure very carefully such things as change in body weight, the initiation of weight loss and look at the shape of those curves and then other parameters that can be measured quite easily now in rodents, both in mice and in guinea pigs.

For screening purposes there really are many indicators of a drug effect. The ultimate endpoint when you are working with rodents, of course, tends to be survival and time to death.

DR. SANCHEZ: I think in looking at a drug the end result is survival, but one shouldn't discount a drug that isn't totally super effective and knocks it flat in its tracks, especially if it has some toxic properties for the patient.

We have said that filovirus infection is a horse race with the virus and the patient trying to mount to an adaptive response that can clear it. If you can buy the patient a little bit of time, that might be enough and in a less toxic way be enough for them to mount a strong immune response and then clear it on its own that way.

DR. BRAY: I guess this is why I'm saying actually weight data. Again, some of that we looked at this morning is surprisingly useful and quite reproducible. Even if a drug doesn't produce survival, if you are comparing a placebo treated or an uninfected animal that there is some protective effect of the drug.

DR. BAVARI: The weight data that Mike actually pointed to us, we weren't doing it until actually Mike said, "This is what you need to do." Actually in the last few years there are really just a few exceptions, animals that are severely losing weight and dying versus animals that are severely losing weight and coming back. I mean, there are some exceptions but in general if they are losing weight they are in trouble.

Maybe that's another way of actually distinguishing. I had a question during my talk about how would you distinguish the top 10 candidates that you have. Maybe that is one way. If they all protect, what else do they do? These parameters are all part of the screening that we're going forward with.

PARTICIPANT: If I can make a comment on an issue that Tony sort of touched upon also. That relates to some specifics of the disease course for the viruses which is very different from a lot of other viral infections that we deal with like chronic infections, HIV, and HCV. This is a very acute disease which is, in fact, a race between the immune system and the virus. All you need to do is to change the dynamics.

The dynamics of this race is different in each animal model. It's different in mouse from guinea pigs, from monkeys, and it will also be different in humans. In each of these animal model that you go in it's a new game with respect to this race and how you can affect that. The drugs do not work just because they completely cleared the virus and result in a sterile situation.

The drugs worked because they changed this curve, the dynamics of the growth of the virus and then the new system can take over. It is, in that sense, somewhat similar to the situation with the vaccines. Because the dynamics is different in the different animals than it would be in humans, what I actually want to point out here is that we should be careful in how extreme we go in determining or setting up all the parameters and endpoints in one animal model or the other because things might be different in humans.

That we will not know until the drug is actually used in humans in future. Basically I think from a practical point of view we will end up taking the few drugs in the end that works best. We cannot artificially set 100 percent survival or what to do if your best candidate has 80 percent survival.

Of course, you take that to the regulatory process and 10 years from now from the field experience we know how it works and that is when we can revisit this issue and then set up some clear parameters of what are those parameters we have to look at for the start of the treatment, what are the endpoints that we should be looking at. I mean, this is all valid and we have to really establish those parameters as much as we can in animals but we have to be aware of these differences, I guess.

DR. JOHNSON: Thank you. Those are some good comments. I think what we'll do now if it's all right with the panel, we are running just a little short of time and the next two questions we've kind of touched upon in our earlier discussions so I thought, if it's all right, we would jump to question No. 5. If there is any time remaining after we discuss No. 5 we can go back to the other two questions.

Question 5 I think was an important one to at least touch upon. The question is what approaches can be used to optimize information collection if outbreaks occur at various times during the development sequence for a candidate product. I guess I would like to sort of say there is kind of two parts to this.

I think one would be what kind of data -- at the present time we really don't have any treatments or vaccines. What type of data may we try to collect from the outbreaks that happen. I think Tony can speak well to the difficulty of doing much but what are some things to maybe consider. Then also if something does become available down the pipeline what might we do with that.

Tony, would you like to start?

DR. SANCHEZ: You're talking about data in the field. Okay. The main suggestion I would have was don't wait until the last minute to figure out what you want to do. I think you need to set up these things well ahead of time.

Target the countries that are mostly likely to have an outbreak, getting contact with health ministries and have all your ducks in a row so that you are ready to go when it does happen. Trying to put these things together and all of a sudden push through these efforts and at times perhaps get in the way of those people who are trying to do their work in the field is problematic. One can do many things but the problem is what will you be allowed to do in this process.

DR. JOHNSON: So I guess what are some of your thoughts? What might you be allowed to do? I mean, are you going to be allowed to do blood draws and take some of that blood and analyze it down the road? Ship it out and analyze it? What are you going to be allowed to do? I realize it's country by country, case by case.

DR. SANCHEZ: To reiterate, could you plug into the system, into the teams that are out there collecting the blood and do the testing there? Are those tests that need to be performed then and there or can they be collected and put into a channel where they can be tested back wherever, at your laboratory or at a site that is in proximity to the outbreak? It all depends on the situation.

One can do a lot of things but the reality of the situation is, as Tom stated, these often occur in very remote areas like in DRC right now getting in and pushing through an effort to collect a certain type of data that might relate to your particular therapy research. It may be possible but you need to get ahead of the curve and plan for it and get everybody on board.

DR. BRAY: I think one of the especially difficult things, I mean, there are so many difficult things about filoviruses but one is probably single cases of infection occur fairly often in Central Africa.

It's just that nobody can recognize them and find them so you have to wait until something big happens such as what is going on right now in DRC to even be able to recognize that this could be a filovirus outbreak and then do some sort of confirmation to say, "Yes, it is a filovirus outbreak."

In terms of preparing and knowing where that next outbreak is going to be, it seems to be a throw of the dice. Up until this present outbreak began I think people would have bet the next ebola Zaire epidemic would be either in Gabon or in Republic of Congo.

Now, it's now in DRC. If you really want to be prepared you would have to have protocols in place and labs and some preparation involving the health ministries of three or four different countries just to have a reasonable chance of being able to do something.

DR. BAVARI: Tony, how would you actually set up a therapeutic protocol? Let's say you have done phase 1 clinical trial. You are satisfied in that small phase 1 clinical trial the TOX data looks promising. What does it take from there to actually set up these type of studies down in DRC or elsewhere? Would you treat everybody who walks in?

DR. SANCHEZ: That's kind of out of my area a little bit. Tom Geisbert, would you like to comment on really the logistics, the issues that one might encounter with trying to do this type of thing? I know it would be possible in Africa but your comments?

DR. KSAIZEK: The one in DRC right now is going to be very sparse. It's going to be difficult to even get the sort of assays we normally field up in operating. It's going to be a pretty big footprint in terms of what they are going to have to project in order to pull that off.

The other issues are there's kind of a bent against research in doing these control efforts so that what they really want are the diagnostic tests done once you've got a patient sort of that has been identified they go into a containment ward if the population is being cooperative.

Maybe if the patient survives they will want another sample drawn to show that the patient has now developed antibody so that they feel comfortable in releasing him back into the community or, at least, into a convalescent ward. That is kind of the nature of these things. Trying to project research protocol on the fly is going to be difficult.

I think what we've tried to do is promote providing some clinical data that the clinicians felt they could use and that promoted blood sample collection in a more frequent sort of serial fashion. More recently, particularly in the Angola outbreak there were hardly any blood specimens collected at all. They got into the business of collecting throat swabs which you can do a PCR on but that is pretty much what you're limited to.

Unfortunately I'm a little bit frightened that has become something that WHO supports and will make it difficult to get something that is not a directed research protocol but rather something that can be set up in a collateral fashion going. I think that is probably the best way to attack this.

The communities are variably cooperative. In some locations like in Gabon and Congo they have been particularly uncooperative even in getting themselves into the isolation wards that are set up so that you get not a lot of patients in a place where you can actually collect specimens from them during the course of illness.

It's difficult but not impossible. With more effort and more people and more resources it might be able to be set up more or less in advance with some contingency protocols, particularly if there was something in it for the National Lab like INRB, for instance, in DRC.

DR. BAVARI: Let me maybe change the question a little bit. How about -- I don't know if there are any requirements here, by the way, or not but what is the actual requirement that HHS or DOD would have for any of these? When do you think that such a protocol needs to be activated? Is it by one case? Is it a single case some place or is it a multiple case? How would you actually activate such a protocol? I don't know if any other requirement people are here or not.

That's another thing actually because that actually has a more direct affect on what we are doing right now in Africa because that is actually going to be a lot harder to do in the clinical studies there in Africa than it is probably done here. I don't know if you want to spend any time talking about that or not.

DR. JOHNSON: I think for the purposes of this question we were considering more outbreaks in Africa. I think what would happen in the U.S. Like you were indicating, that's a policy level decision that is at least above my head.

DR. BAVARI: It's above mine. If they increased my salary twice I would be able to answer it.

DR. SANCHEZ: I think fear is a great motivator in a situation like this. In Africa, in Uganda, while we were there the first publication of a protective vaccine in nonhuman primates monkeys came out and they were questioning, "Why didn't you have the vaccine here?" They were ready to take it then because they were at that point so afraid of what was going on they were looking for anything.

PARTICIPANT: Relevant to the issue -- excuse me. Sorry. Relevant to the issue of outbreaks here, has any modeling been done in terms of the increase in numbers of BL-4 labs and increased exposure of laboratory workers to filoviruses and what the expected rate of inoculations might be and whether or not when therapeutics and vaccines get developed whether we can be prepared to use them in those circumstances? Is anybody aware of that?

DR. SANCHEZ: I'm not up on the square footage that is going to increase but I think in the next few years it's going to quadruple. The amount of nonhuman primate work is going to increase in other models. Yes, it's going to be an issue and it needs to be taken a look at.

DR. BAVARI: Actually I don't know if there are a lot of -- is it a holding facility at CDC like USAMRIID has a patient holding facility?

DR. SANCHEZ: No, we have an agreement -- well, Tom can address.

DR. KSAIZEK: We have a contractual agreement with the hospital. The CDC recommendations for handling patients whether they be imported or from labs is that they don't necessarily have to go in the slammer. We certainly have no objection to you trying to maintain a slammer, although I'm not sure that is your policy any longer but rather to take care of them in facilities that have been pre-setup with negative airflow.

There is not even a requirement for HEPA filtration, although I've seen one publication that seems to suggest that somebody made that decision for us. People are aware. I mean, there's sort of a committee preestablished to help govern it but a lot of this is done on the fly.

We all keep our fingers crossed and depend on having experienced personnel guiding and training the new personnel in the existing facilities. The equation where there is a lot of new facilities and the culture has to get started again is a little bit different situation.

DR. SANCHEZ: For those of you who are unfamiliar with the slammer, it's a kind of a hospital room jail cell that they keep people who get stuck and can monitor their progress.

PARTICIPANT: I was curious to know if any drug like Xigris or something similar has been used for ebola infections? Can you set up something in an outbreak area that you can try something, a drug that is already in use in humans for another use could be tried in this?

DR. BRAY: I think you would clearly have to have a clinical protocol. I don't think there is any way, particularly anything that is funded by NIH, directly or indirectly, would require a protocol approved by a recognized ethics committee. In fact, one that meets the standards of HHS in terms of composition and the function of that committee. Anything of that sort requires a lot of preparation.

There has been interest because outbreaks in Central Africa are so sporadic and unpredictable maybe there should something like a regional IRB, an institutional review board, that could review protocols across several countries. I don't think there's been any progress with developing that.

PARTICIPANT: Understanding that it is difficult and we would have to have protocols and these are sporadic outbreaks and it's very difficult to control. I think what I would like to hear is if you could figure out how to get samples from an outbreak situation what samples do you think we should be going after?

DR. SANCHEZ: At least blood and processing of PBMCs is a good idea as a minimum. From there it becomes problematic performing liver biopsies and things get more complicated, more dangerous. You have difficulty taking specimens from fatal cases because of the social problems there. The facilities won't let you. It will be difficult to get all the specimens one would like but it's possible to get blood.

DR. BRAY: I guess I would say right now it seems to me that we have the best shot really at developing post-exposure prophylaxis. We've got a number of approaches that work pretty well in rodent models and nonhuman primates. Ideal specimens in my view are those that could tell you something about how people are becoming sick.

If there is a known exposure, for instance, during an outbreak setting if it's possible to collect blood samples on someone who hasn't already shown up sick but is during the incubation period that would be extremely useful.

DR. GEISBERT: I just want to address Pat's question. My understanding is that Xigirs is already licensed by Lilly for severe sepsis so a physician could, it's my understanding, use that off label and maybe some of the regulatory folks want to comment to Pat on that but that was my understanding.

DR. SANCHEZ: What was that drug?

DR. GEISBERT: Xigirs, activated protein C release drug. I think that's what you said. Right, Pat?

DR. JOHNSON: We probably have some FDA colleagues who could comment on that.

DR. STYRT: FDA doesn't regulate the practice of medicine by individual practitioners in terms of the off-label use of an established drug. There are differences in how these things are handled when it's a matter of a Government agency specifically releasing a product for a specific investigational off-label use.

There are also obviously differences in terms of whether someone interested in developing an approved product for a new indication has an interest in learning something more about how the product works and there, I think, is where your issues about setting up protocols and setting up studies become additionally important.

There are a lot of differences in terms of the individual situation. Obviously if you are talking about an individual physician who happens to have the drug in a country other than the U.S., then regulatory issues may not be very relevant unless -- U.S. regulatory issues may not be so relevant there but discussion of U.S. regulatory issues can still be important if you are thinking about trying to establish efficacy and get the product potentially approved for the new indication at some time in the future.

My suggestion would be that this kind of approach is, again, something that can be relevant and potentially important to talk with the FDA about if there is someone who is interested in potentially sponsoring and learning about a new type of use and that may be a separate issue from the issue of whether you are required to have an IND protocol which depends on licensure status on who is doing what with the product and where they are doing it.

PARTICIPANT: How much usable data can be recovered from less invasive samples like nasal swabs, throat swabs, stool samples in terms of disease progression? Is there any knowledge about that?

DR. SANCHEZ: Not much. I guess from stool and nasal one might take a look at IgA and virus load and try to relate that to disease severity and just responses by the patient but not much.

DR. REED: I think you also need to -- I was just thinking if there is animal data and we can obviously say some things like the D-dimers there in that case gives us some ideas of things to look at but you are also limited in what you can draw for your blood samples and that's going to drive what you look at as well.

PARTICIPANT: I want to make two quick comments. First, in light of what Barbara said earlier and also the discussion about the models today. Point one has to do with the issue of what would be monitored in the patient who is treated with a potential antiviral compound. What we learned yesterday about the discussions about vaccine was obviously that we need to develop very careful immune correlates of vaccinations.

I say that because any of the potential kinds of compounds that come out of -- let's say they are very potent antifiloviral agents that come out of a screen. If we don't understand carefully how they work, then it won't be possible to have a parameter to measure in the host no matter how efficacious they are.

I guess I might say then broadly in the Government's approach to having RFAs and UR01s to develop antiviral compounds if that doesn't go along with appropriate resources to understand how the virus replicates and interacts with the host. It may be possible to have a situation where you have a compound that works perfectly well but you don't actually have a really good way to monitor or identify its target.

The second point would be relevant to point 4 which is the inhibition of virus replication. That is any inhibition of the virus polymerase would be a wonderful target. Perhaps the only target that wouldn't require the compound to be targeted toward the host but actually could be targeted directly toward the virus. There really needs to be a lot of -- it would seem to be a very area or avenue of future research in developing inhibitors.

I don't know if this is a formal policy but it certainly has been a tacit policy that research on preliminaries has been limited by the potential concerns about spreading the preliminary gene around because of a potential safety bio-terrorist access reasons. That may have been valid maybe 10 years ago when clay gases synthesis technology was relatively unknown.

Now that the polymerase sequences are all published and the technology is widely available, it's really more impedes research rather than makes us safer to limit the access to the preliminaries. There really needs to be a lot of work done on the preliminaries as a target for antiviral therapies. I'll make those two points.

DR. SANCHEZ: I agree with your second point, the preliminaries that you could synthesize that easily on your own. It's not a problem. Your first point regarding the ability to identify exactly how an antiviral is working may be easy in terms of siRNA and those types of approaches. With certain other compounds that have a broader effect and affect the virus through an assortment of pathways may not be easily defined.

For example, in the virus entry work that

I have done, I have seen that chlorpromazine affects virus replication but it doesn't have any specific affect in the cell. It has a broad affect so that may be troublesome in trying to nail down exactly what's going on in an animal model.

DR. JOHNSON: I see that we are out of time so I would like to thank all of the panel members.

DR. BAVARI: I think there is one more question.

DR. JOHNSON: Sorry.

PARTICIPANT: I will try to make this short. Is there any utility in transgenic animals for testing host structured therapeutics, specifically human gene sequences or specific human engines that may not be homologous in the existing animal models.

DR. BAVARI: Definitely, yes. Definitely. If you actually can have a model that mimics the nonhuman primate as Tom described, the fibrin deposition specifically following ebola infection, I think that would be pretty ideal in a small model. That's smart actually.

DR. REED: I think the point has been made, too, that not every study is necessarily an animal rule study. It provides you information about the disease or the progression or how your therapeutic is going to work. That is still valuable information that can be used in getting that drug or vaccine to licensure.

DR. JOHNSON: All right. I would just like to thank the panel again for all their effort and for the really good discussion we had today. I would like to thank everybody who stuck through to the end. Thank you very much for your attendance. I hope that everybody found this discussion useful. I wish everybody a safe trip home. Thanks a lot.

DR. CHEN: I just have one thing to say. I got a lot of questions about requests to have the presentations be accessible to all the participants so we are going to put the presentations on the website. If you have registered or you are on the participant list, you will get a notice when it's up on the web. Thanks for everybody coming for this meeting and to stick to the last minute.

(Whereupon, at 12:33 p.m. the meeting was adjourned.)